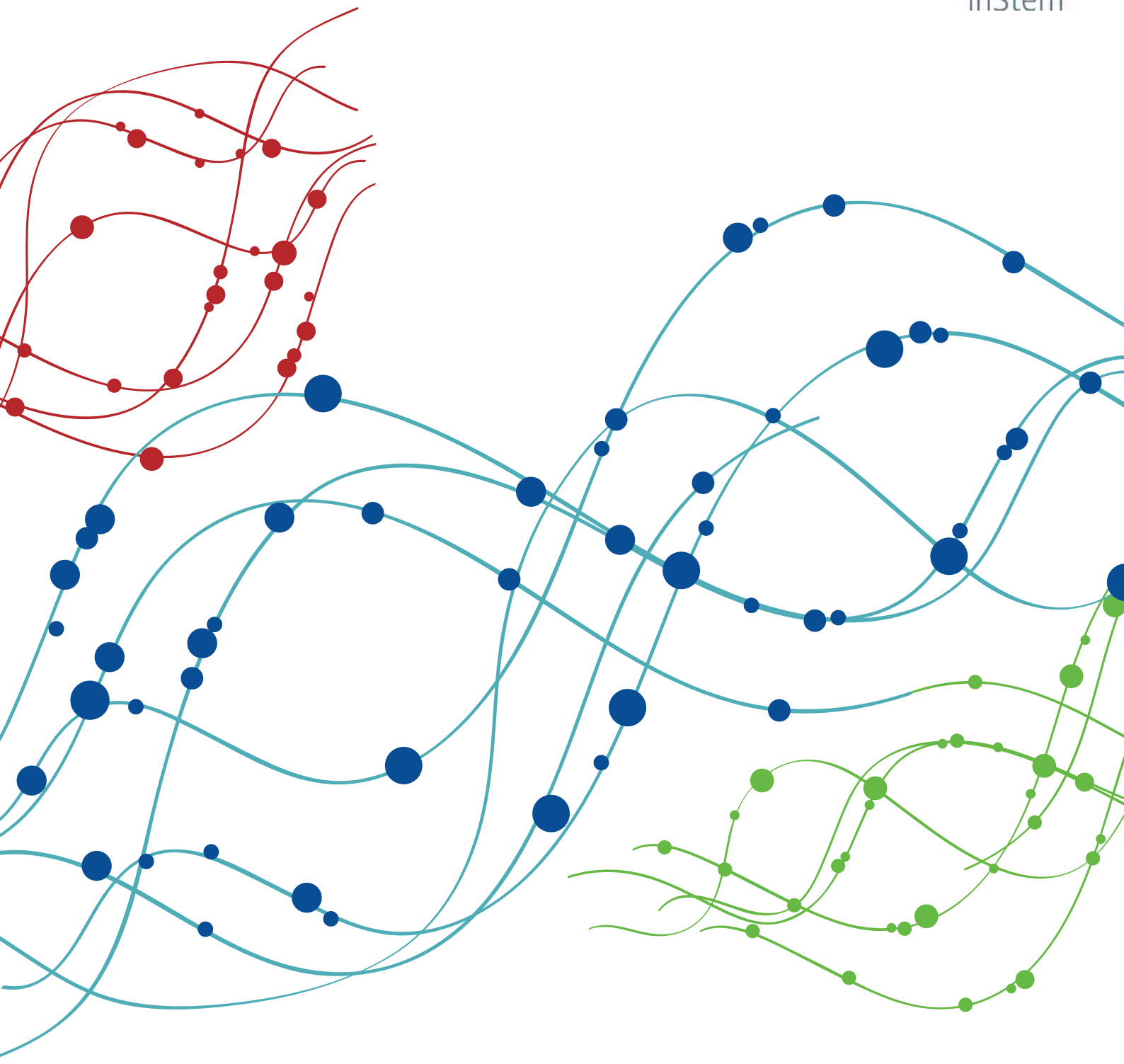


inStem

Annual Report | 2012-2013

Institute for Stem Cell Biology and Regenerative Medicine, Bangalore

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

Contents

S. Mayor

Director's Note

inStem is four! Sometime in January 2013, Prof. VijayRaghavan, the former director of National Centre for Biological Sciences (NCBS) and founding director of inStem, went missing. To our astonishment he surfaced in the Capital, anointed as the new Secretary of the Department of Biotechnology. Here's wishing him all the best in his new job, and of course the very best in the service of funding the best of biological sciences in this country. It is heartening that someone of Vijay's calibre as a scientist and administrator (much celebrated by his recent recognitions: Elected to the Fellowship of the Royal Society and the Padma Shri award by the President of India) is not only nominated for the job, but accepts the challenge. Much as we thought that Vijay would buckle under the immense pressure of juggling so many balls that he had up in the air in Bangalore, he has gone and added a huge ball to his juggling list. Happy juggling, dear comrade!

We also thank Vijay for his inspiration, and the rousing start that he has given inStem. To reiterate a key tenet of the founding charter- 'inStem will create a focus (or foci), and scientific programmes centred around on understanding human cell differentiation, tissue formation and regeneration, and apply its discoveries in the exciting frontier of regenerative medicine.' It is also clear we are not the only ones thinking of such goals.Therefore, to succeed, an institutional culture that is different from current ways of functioning must be developed. inStem envisages 'a collaborative institution for scientific discovery where we pool intellectual and physical resources in an interactive collegial environment that allows investigators to work in interdisciplinary teams. Such a culture will allow inStem to tackle major, complex, scientific problems that are difficult to pursue in single investigator laboratories'.

Given the environ we find ourselves in, with NCBS as an institutional model with an excellent track record, painstakingly achieved over the past twenty some years, we cannot afford to look for success with just more of the same. We must have a measure of the difference that doing science at inStem can bring. In realising the spirit of the founding charter we have a good metric; and here at the young age of four we would have to say that all systems are ready to go, and there is huge potential. If we are ready to measure ourselves we must also learn to take on board criticism, as it surely must arrive if we are doing anything different (and meaningful).

We now have four themes firmly in place, which embody this spirit; the Centre for Tissue Inflammation and Homeostasis (CITH); the Centre for Brain Development and Repair (CBDR);the Centre for Cardiovascular Biology and Disease (CCBD); the Centre for Chemical Biology and Theraputics (CCBT). In addition, we have a gregarious Technology Team (who call themselves the TAS team) who are trying to push and pull advances in technological innovations that could help catapult the collaborative space emerging at inStem. The next few years should see us building on the strengths of the ongoing programme on Stem Cell Potency (SCP) to attract a new theme(s) centred around questions about evolutionary diversity of stem cell mechanisms, and the physical and chemical biology of stem cells, as well as others, that we see enhancing the interdisciplinary programmes at inStem. This year, we have also forged greater links with the clinical translational unit run by Alok

Srivastava at the Christian Medical College (CMC)'s Centre for Stem Cell Research (CSCR). Many of our themes will soon harbour colleagues from Vellore as collaborators or core theme members, clinical or otherwise, and vice versa.

It has also been a good year for realising funds and some exciting science. The CCBT programme, long in gestation, has now been allocated funding from the DBT. Under the able stewardship of Ashok Venkitaraman from the University of Cambridge, the years ahead look very promising. The CBDR programme is being considered for funding and nicely draws on the skills of our collaborators (Siddharthan Chandran and Peter Kind, as well as Adrian Bird and Richard Morris) from University of Edinburgh and with our colleague, Sumantra Chattarji (NCBS) jumping into the fray to direct this centre, the CBDR theme looks nicely poised too. Continued extramural support from the Shanta Wadhwani Foundation for the CBDR and CCBD themes is also gratefully acknowledged since this has allowed us to explore daring paths, and not just the well-trodden.

The collaborative environment at NCBS, Centre for Cell and Molecular Platforms (CCAMP) and ourselves, united along with the University of Agricultural Sciences (UAS) under one umbrella called the Bangalore Biocluster, now boast of over 50 laboratories focusing on biology from the single molecule to ecosystems. Advanced technology centres and infrastructure for high throughput experimentation, and reduced barriers to tackling experimentally challenging questions is the need of the hour. Combine this with a campus-wide international graduate and post-doctoral fellows programmes we have a fair chance at reasonable success.

A great challenge ahead lies in building an environment that respects individual creativity, accomplishment and endeavour, and at the same time recognising and nurturing the heady atmosphere of a collaborative space, vital for taking on scientific questions that a single laboratory will not be able to address. This is the identity that we hope inStem will differentiate into, distinguishing itself from all other institutes for basic biology.

inStem is now being run with the help of a very active Steering Group who provide regular counsel. This includes the Deans of inStem (Jyotsna Dhawan and S. Ramaswamy) and NCBS (Upinder Bhalla) as well as Apurva Sarin and Sumantra Chattarji from NCBS. We also have a revamped governing council and a scientific advisory board. I take this opportunity to welcome our new colleagues on board, and thank those who have remained for agreeing to continue to guide us. I would also like to take this opportunity to thank our outgoing Board members for their extraordinary support. We are also particularly pleased to report a tremendous sense of enthusiasm amongst our colleagues at NCBS and CCAMP in support of our experiments in building new institutional identity, culture, and scientific programmes. The next year will see our collective vision unfold, and the future appears unreasonably inviting, if I may say so.

Administration Report

Expansion, collaboration, and consolidation

T.M. Sahadevan, Head Administration and Finance

The year has been the most happening year in terms of the number of new Principal Investigators joining inStem. This was made possible by the availability of space at the beautiful new NCBS Southern Laboratory Complex, so that inStem could occupy a large laboratory area covering one entire floor (~40,000 sft). Here, inStem has set up a state-of-the-art stem cell laboratory, which is now fully functional and scientists (both short- and long-term) have set up their research activities. The laboratory has an open lab design encouraging informal interactions between areas and groups, and reflecting the collaborative philosophy that led to the founding of inStem, with deep ties to NCBS. The other notable feature has been approval by the Dept. of Biotechnology of a Centre for Chemical Biology and Therapeutics, as an extra-mural grant.

In terms of infrastructure, construction on campus is proceeding briskly, which includes the guest house, hostel, residential quarters, and a dining block. We are close to issuing the work order for the main construction activity – the inStem Main Laboratory. Though the master plan was approved by the Governing Council in March 2012, this had to be revised as the last stretch of land allotted was found to be in a Biodiversity Heritage zone, and had to be swapped in November 2012 with another stretch of land. Thus, the master plan had to be redrawn. This was approved by the Governing Council in November 2012. Tender documents have been issued to the six shortlisted Contractors for the Main Laboratory Block; thus it is hoped that the work order for this major construction is likely to be issued soon.
The massive support from NCBS in all matters including Administration is gratefully acknowledged.

The following international conferences were organised during the year:

- International Stem Cells and Regenerative Medicine Conference, jointly organised by inStem (Bangalore, India) and the UCL Centre for Stem Cells and Regenerative Medicine (London, UK) held at UCL, July 11-13, 2012.
- Course on Membrane and Membrane Associated Proteins, August 13-17, 2012.
- inStem Mouse Embryology Workshop: Stem Cells to Organogenesis. Symposium: March 10-12, 2013 and Hands-on Training Workshop: March 13-23, 2013.

As in previous years, Young Investigator Meeting was held this year as well (Jodhpur, February 2013) and conducted jointly by many institutions/departments including the Departments of Biotechnology, and Science and Technology. An MoU for increased collaboration and sharing of facilities between the Cluster institutions (inStem, NCBS, and C-CAMP) with the University of Agricultural Sciences (in whose larger campus the Cluster is located) and to constitute a Coordination Committee is in final stages of discussion. We look forward to greater connectivity between these components in the coming years, enhancing the concentration of research and academic activities.

2013:
A year of change at inStem

Prof. K. VijayRaghavan, inStem's founding Director was appointed Secretary, Department of Biotechnology, Govt. of India in early 2013. In January 2013, Prof. VijayRaghavan was awarded the Padma Shri, in recognition of his services to developing the growth of and promoting excellence in biological sciences in India. This award is the latest in a long list of laurels for Vijay, which includes being elected as a Fellow of the the Royal Society, London in 2012, in recognition of his research in biology of muscle and neurons in Drosophila, and his scientific leadership in growing and building insitutions of exellence. Prof. VijayRaghavan's new challenges as the head of a national life science funding agency will include boosting Indian efforts and linking to global initiatives. We hope these responsibilities will be offset by a continued association with the vibrant collaborative campus that he helped create.

Prof. Satyajit Mayor was appointed Officiating Director of inStem in March 2013. Prof. Mayor is also the Director of the National Centre for Biological Sciences. Prof. Mayor received his Master's degree in Chemistry from the Indian Institute of Technology, Mumbai. He then went on to do a Ph.D. in Life Sciences at the Rockefeller University. He started his own laboratory at NCBS in 1995. Prof. Mayor is the recipient of several national and international awards such as the Infosys prize in Life Sciences, Wellcome Trust International Senior Research Fellowship, Swarnajayanti Fellowship, Shanti Swarup Bhatnagar Award, and the JC Bose Fellowship.

The broad aim of Prof. Mayor's laboratory is to provide an understanding of the molecular mechanisms of endocytosis in metazoan cells, and study this phenomenon at many scales. At the molecular scale his group works to uncover the molecular players in endocytic processes; at the mesoscopic scale research in his laboratory attempts to provide a physical description of cell membrane structure and organisation process and its material properties; at the cellular scale the work is aimed at synthesising a role for endocytosis in cellular signalling and cell surface homeostasis; at the scale of the tissue the group wishes to determine how control of endocytosis impinges on many developmental programmes in tissue morphogenesis.

Prof. Mayor assumes his role as inStem's officiating Director at a time of growth and consolidation for this fledgling institute-exciting times lie ahead both in scientific and academic programmes with a number of international collaborative initiatives underway.

Core Scientific Activities and New Initiatives:

An Overview of 2012-2013

Our scientific activities over the past year have been focused on restructuring and strengthening themes established last year and developing new programmes in two new areas. The change in organisation reflects the process of evolving streamlined mechanisms that explore new interfaces between existing programmes.

Broadly, three areas of activity have emerged:

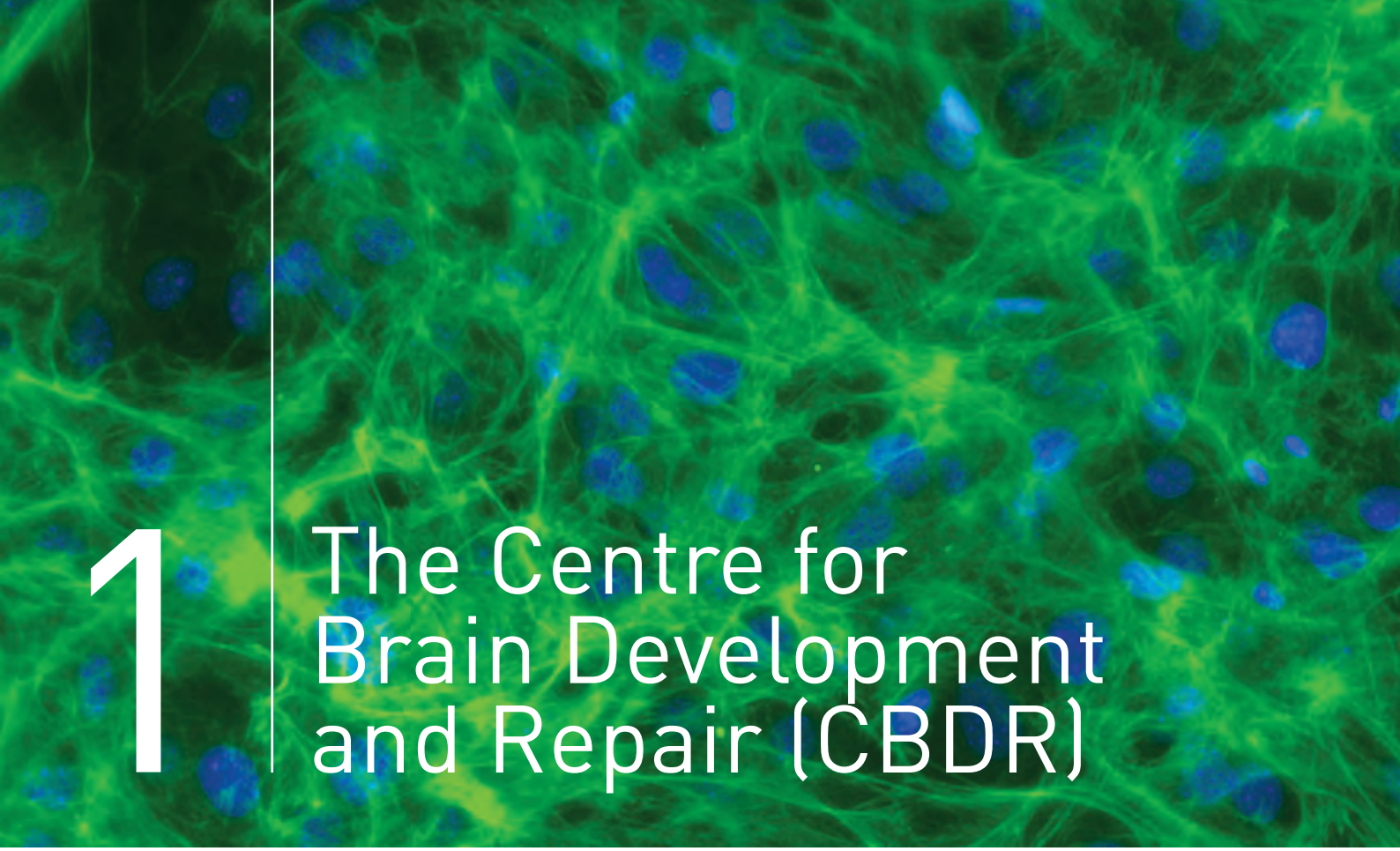
- A set of five research themes aimed at understanding and harnessing stem cell function towards new basic knowledge and translation. The themes in this area include

  - Centre for Cardiovascular Biology and Disease
  - Centre for Inflammation and Tissue Homeostasis
  - Centre for Chemical Biology and Therapeutics
  - Centre for Brain Development and Repair
  - Programme on Adult Stem Cell Potency

- A diverse group with a range of strengths focusing on the development of purpose-built technologies ranging from new sensors for detecting sub-cellular events to new materials for drug delivery to new approaches in genome-wide interrogation of stem cell properties. This team is called Technologies for the Advancement of Science and aims to convert gaps in knowledge into opportunities for innovation.

- An integrated group of basic scientists and clinicians focusing on translational aspects of stem cells at the Centre for Stem Cell Research, CMC Vellore. These programmes range from basic investigations of iPS technology to preclinical gene therapy models and clinical translation of hematopoietic and endothelial cells.

The following scientific reports reflect the philosophy of team-driven scientific endeavour, focusing on the overarching goals of the theme, involving the strengths of each of the participants.



1 The Centre for Brain Development and Repair (CBDR)

The Centre for Brain Development and Repair (CBDR) is a collaborative programme between inStem and the University of Edinburgh (UoE) focusing on autism spectrum disorders (ASDs) and intellectual disability (ID). To this end, CBDR brings together a range of expertise in several fields of neurobiology including neurodevelopment, synaptic function and plasticity, human stem cells, and cognition-behaviour.

Disorders of the brain represent a growing and major public health threat to India. Although these are a disparate group of currently untreatable conditions that include acquired, developmental, and ageing-related diseases there are nonetheless common themes and needs. These include the recognition that key molecular mechanisms and biological processes are shared across many of these diseases indicating that discoveries in one disease group will inform the other and vice versa. Uniformly the unmet need is for a human-based and human-led approach to investigating the cause(s), consequence, and ultimately treatment of these diseases. Critically this requires a cadre of neurologist/scientists who can both inform the basic science as well as lead the underpinning clinical research necessary for translation of laboratory discoveries. Presently India does not have such a group of physician-scientists. CBDR will address this major gap in Indian translational medicine by adopting a targeted approach focusing on ASD/IDs. The programme fully appreciates that other brain conditions rooted in environmental causes are arguably equally pressing e.g. infectious or trauma-related disorders. However, the team believes that ASD/ID not only represents an area of major need but also constitutes a field in which we are well-positioned on grounds of both the research excellence of our team and the rapid rate of genetic discoveries to make clinically relevant observations faster than for other brain diseases.

THEME MEMBERS

Sumantra Chattarji
Theme Coordinator
Professor of Neurobiology at NCBS
His group is studying the neural basis of the cognitive and emotional symptoms of Fragile X Syndrome (FXS), the leading genetic cause of autism and intellectual disability. His l ab's work has shown that it is possible to reverse or prevent many of the synaptic and behavioural phenotypes of FXS using pharmacological and genetic rescue strategies in mouse models.

Siddharthan Chandran
Collaborative Science Chair, MacDonald Professor of Neurology, Head of the Division of Clinical Neurosciences & Director of the Centre for Clinical Brain Sciences, University of Edinburgh
He works in the emerging discipline of Regenerative Neurology and interested in neurodegeneration, stem cells, and brain repair. His research combines laboratory and clinical activity that includes human stem cells and specialist clinics to both study disease as well as undertake early phase clinical trials.

Peter Kind
Collaborative Science Chair, Professor of Developmental Neurobiology & Director of the Patrick Wild Centre, University of Edinburgh
His lab studies the interaction between genetics and environment in the development of neuronal circuits. More specifically his lab examines the synaptic basis of neurodevelopmental disorders with specific focus on ASD/IDs.

The goals of CBDR are:

Programme 1.
Modeling human ASDs 'in a dish': Human discovery and testing platform
Use human pluripotent stem cell-based *in vitro* systems for both scientific discovery of cellular and synaptic mechanism underlying ASD/ID and for subsequent large scale screening for effective pharmaceutical compounds.

Programme 2.
The Autistic Network: From Pathways to Rescue
Generate new mouse models of highly penetrant single gene causes of ASD/ID to effectively model autistic and cognitive behaviours that can accurately reflect autistic features in humans. These models permit the use of functional MRI in awake, behaving animals that then allows parallel studies in mice and humans using the same modality; allowing, for the first time, human clinical trials to be informed by comparable trials in mice.

Programme 3.
Autistic Function: Mouse Models for Fundamental Discovery and Translation
Test convergence of developmental and cellular phenotypes associated with genetically heterogeneous causes using mouse models of highly penetrant single gene causes of ASD/ID. The goal is also 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.

RECENT PUBLICATIONS

- Muddashetty R, Nalavadi C, and Bassell GJ. Fragile X syndrome: A disorder of synaptic protein synthesis dynamics. IISc J (2012) 92 (4): 447-464.
- Wijetunge, L.S., Chattarji, S., Wyllie, D.A.J., and Kind, P.C. (2012) Fragile X syndrome: From targets to treatment. Neuropharmacology doi.org/10.1016/j.neuropharm.2012.11.028.

HONOURS AND AWARDS

Debarati Mukherjee - Wellcome Trust/DBT Early Career Fellowship (2012)
Reena Rathod - DBT-Research Associateship in Biotechnology & Life Sciences (2013)

INVITED TALKS

Sumantra Chattarji
- Gordon Research Conference on "Fragile X and Autism-related Disorders", Stonehill College, MA, USA (2012)
- Molecular and Cellular Cognition Society (MCCS)-Asia Symposium, Kyoto, Japan (2013)

Ravi Muddashetty
- National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India (2012)

- Centre for Neuroscience, Indian Institute of Science, Bangalore (2013)
- Sastra University, Thanjavur, India (2013)

Archana Purushotham
- Narayana Institute of Neurosciences, Bangalore, India (2013)
- Mahatma Gandhi Medical College and Research Institute, Pondicherry, India (2013)

TEAM MEMBERS

Sumantra Chattarji: Debarati Mukherjee (post-doctoral fellow)
Ravi Muddashetty: Reena Rathod (post-doctoral fellow), Vishal Tiwary (PhD student), Aswini Krishnan (JRF), Preeti Kute (JRF).

COLLABORATORS

Ravi Muddashetty: Prof. Poul Hyttel and Prof. Jan Gorodkin (University of Copenhagen).

Archana Purushotham: Prof. Vinod Menon, Stanford University; Dept. of Radiology, HCG Bangalore Institute of Oncology; Prof. Andrew Stanfield, Patrick Wild Centre, University of Edinburgh; Ravishankar Pervaje, Sushruta Ayurveda Hospital, Puttur; Maarten Lansberg, Stanford Stroke Centre; Dept. of Neurosciences, Bhagwan Mahaveer Jain Hospital; Sanjeev Jain, Professor of Psychiatry, NIMHANS; Academy for Severe Handicaps and Autism (ASHA), Bangalore.

Ravi Muddashetty
Senior Fellow
Studying the mechanism of activity mediated protein synthesis and its implication for neurodevelopmental disorders. Primary focus of current research is to identify microRNAs involved in FXS and elucidate the link between translation dysregulation to FXS phenotype.

Archana Purushotham
Senior Fellow
Using a combination of neuroimaging and clinical studies to better understand brain dysfu nctions in stroke and autism spectrum disorder.

2 Centre for Cardiovascular Biology and Disease

THEME MEMBERS

James A. Spudich
Theme Coordinator
He is a Collaborative Chair at inStem and is the leader of the Cardiomyopathy project. At present, Spudich is the Douglass M. and Nola Leishman Professor of Cardiovascular Disease in the Department of Biochemistry, Stanford University School of Medicine. He has been awarded the 2011 Lasker Award.

The Cardiomyopathy group is focused on inherited cardiomyopathies to better understand cardiovascular disease. Its primary interest is to understand how changing a single amino-acid residue in a sarcomeric protein (myosin, actin, tropomyosin, or the troponins) causes inherited primary dilated or hypertrophic cardiomyopathy, which affects 1 in 500 people. The programme aims to group the mutants mechanistically as an initial step in developing small molecule treatments.

Initially focusing on alpha-tropomyosin, the approach is to detect functional changes using binding and fluorescence assays, *in vitro* motility assays, laser trapping, and single-cell contractility assays. Since a single amino-acid change is sufficient to cause disease and human and mouse sarcomeric proteins tend to have multiple differences between them, the programme is humanising the mouse cardiac sarcomere to develop better animal models.

The goals of the Cardiomyopathy programme are:

- Understanding biochemical/biophysical mechanisms underlying cardiomyopathies
- Understanding cellular mechanisms underlying cardiomyopathies
- Developing small-molecule treatments for cardiomyopathies (long-term)

Progress and Ongoing Work:
Biochemical, biophysical, and cellular mechanisms underlying inherited cardiomyopathies

In this aim, the programme proposes to detect mechanisms shared between multiple cardiomyopathy-causing mutant sarcomeric proteins. Put less formally, the goal is to divide up the mutants into 'biochemical buckets' based

on the mechanisms of disease. This has already been demonstrated in the Spudich lab with the analysis of the first two beta-cardiac myosin heavy chain mutants. One (R453C) is associated with increased force production without the usual changes in calcium sensitivity. The team will concentrate on those mechanisms which are amenable to interventions, primarily with small molecules.

John A. Mercer
Investigator inStem
His group at inStem is trying to understand inherited cardiomyopathies at multiple scales of organisation. Primarily investigating how single amino acid changes in sarcomeric proteins cause cardiomyopathies.

Initial efforts have been devoted to tropomyosin mutants, complementing the Spudich lab's study of beta-myosin heavy chain and troponin mutants. Since the regulation of muscle contraction by calcium is dependent on modulation of the azimuthal movement of tropomyosin along the surface of actin to allow myosin binding, we have concentrated on simple *in vitro* binding assays, which to the team's surprise had not been performed for all known mutants to date. While this necessarily reproduces some existing data, it provides a solid foundation for future studies. Preliminary data on tropomyosin mutants E54K, E62Q, K70T, E180G, E180V, L185R, D230N, and M280T suggest that even at the level of simple binding of tropomyosin to actin, mutants with the same phenotype have multiple molecular mechanisms for causing cardiomyopathy. The team is collaborating with R. Sowdhamini of NCBS to study the structural changes caused by these mutations.

Additional biochemical/biophysical assays will be chosen to complement ongoing work at Stanford and availability of appropriate resources in the Bangalore Biocluster. The first assay measures the inhibition by tropomyosin of myosin S1 subfragment binding to pyrene-labelled actin. The second assay uses fluorescence to measure conformational changes caused by calcium binding to reconstituted thin filaments that include troponin C labeled with 2-((4'-iodoacetamido)anilino)-naphthalene-6-sulfonic acid (IAANS), to measure changes in calcium sensitivity. This assay has been used in the Spudich lab and their data show that it gives pCa curves almost identical to those obtained with ATPase assays, which are much more temperamental. The third is the *in vitro* motility assay, invented in the Spudich lab. Finally, for any cases that warrant it, we can perform laser-trap measurements of force production at Stanford.

A rapidly growing facet of the field is measurement of the effects of disease-causing mutations on single-cell contractility. Many methods are being developed for doing this, including traction-force microscopy, deflection of micropillars, and deflection of carbon fibres. The team proposes to perform these assays using cardiomyocytes from multiple sources: differentiated from patient iPSCs, differentiated from mutagenised human PSCs, and the ESCs used to produce the humanised mouse models described below.

The laboratory of our inStem colleague Kouichi Hasegawa will generate cardiomyopathy patient-derived iPSCs from CD34-positive cells from peripheral blood. The team will also collaborate to generate knock-in hPSC lines with point mutations in cardiomyopathy genes with TALEN gene-targeting technology. These will be differentiated into more mature cardiomyocytes than otherwise available using an efficient protocol with chemical compounds transferred from Kyoto University, where Norio Nakatsuji of iCeMS is also a collaborator. These efforts will generate

an unlimited supply of cardiomyopathic cardiomyocytes. In collaboration with Namrata Gundiah of IISc, the programme has set up a traction-force microscopy using both PDMS and polyacrylamide substrates. Her laboratory is also fabricating micropillar arrays.

Currently, the team is close to performing its first differentiations from mouse ESCs. No group to date has published data showing mature, rod-shaped morphology from ESC or iPSC-derived cardiomyocytes. For this reason, the differentiation state of our cardiomyocytes will dictate our method of measurements of single-cell contractility. The team is most likely to employ traction-force microscopy on PDMS or polyacrylamide substrates, as micropattern printing of extracellular matrix proteins on them has been shown to dramatically induce the anisotropic orientation of sarcomeres.

A humanised-mouse model

To produce mature cardiomyocytes and approach the organismal level, we have begun work on a humanised-mouse model to overcome problems with existing mouse models. While many transgenic models have been produced, all suffer from a major caveat: the fact that a single amino-acid residue substitution is sufficient to cause disease, while at the same time, many of the sarcomeric proteins differ by dozens of residues between mouse and human. Moreover, the predominant adult ventricular cardiac myosin heavy chain differs: in mice, the alpha form dominates, while in humans, the beta isoform dominates.

As the transgenic facility in the Bangalore Biocluster is not yet functional, the ESC and mouse work has been outsourced. The scheme for the whole-locus knock-in is shown in the accompanying figure. Briefly, all exons and introns of the human beta-myosin heavy chain locus (MYH7) have been inserted into the alpha-myosin heavy chain locus (Myh6). We hypothesize that when bred to homozygosity, this knocking will provide a mouse heart in which human beta-myosin heavy chain is the dominant ventricular isoform. Then cardiomyopathy-causing mutations will be made in MYH7 using TALEN technology.

ESCs with the humanised allele have been produced and shipped to us for *in vitro* differentiation. They also have been injected with blastocysts to produce chimeric mice, which have transmitted the humanised allele through the germline. Heterozygous mice with only one humanised allele are healthy at eight weeks of age. In collaboration with Leslie Leinwand of the University of Colorado, the team has demonstrated that they express the human protein at ~40% of the level of endogenous mouse alpha-myosin heavy chain. As this judgment is based on only the migration of a band on a gel, we have confirmed that it is the human protein by mass spectrometry.

Histological and stress analyses of both heterozygous and homozygous mice will be performed after generating a breeding stock. Next TALEN genome editing technology will be used to insert the disease-causing mutations that most interest us into the humanised gene. This is a very exciting development, as it has the potential to provide new, and better single-cell and animal models.

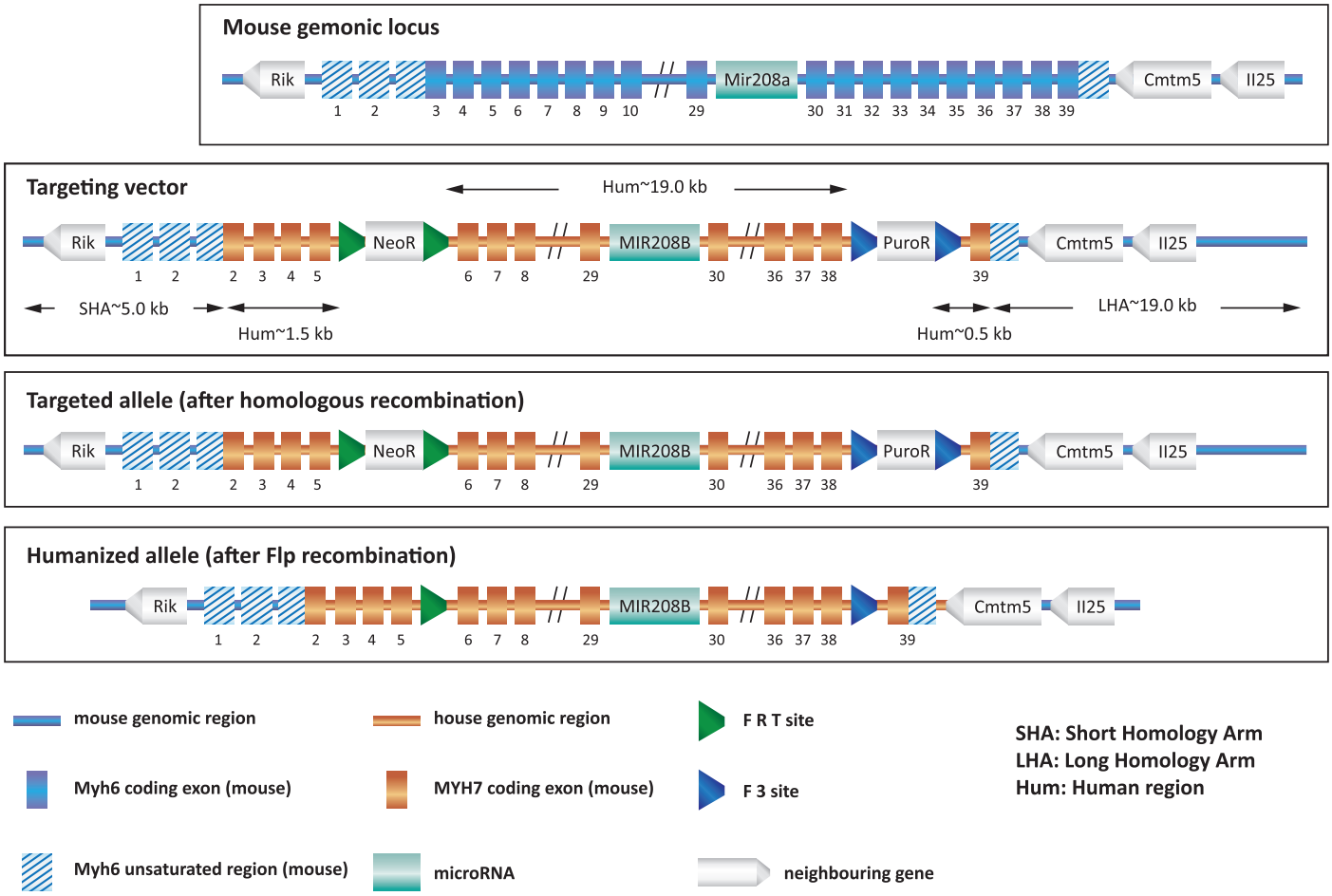


Figure 1: Humanisation strategy for substituting the predominant adult human cardiac myosin for the predominant mouse cardiac myosin.

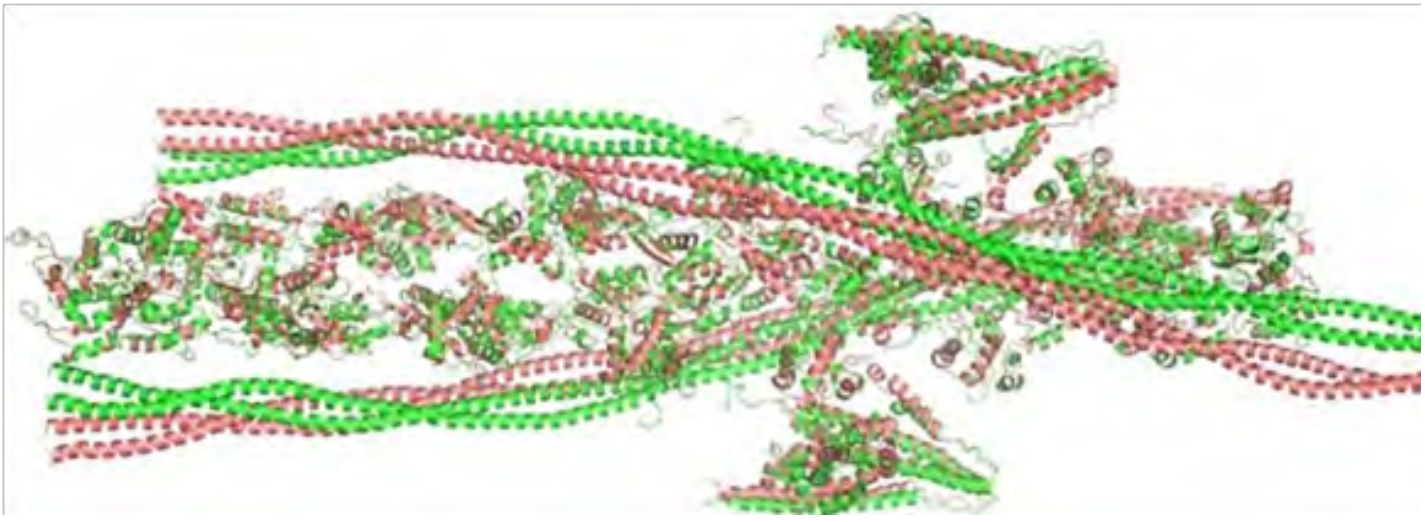


Figure 2: Model of the thin filament (actin, tropomyosin, and troponin) in the blocked (green) state in the absence of a calcium signal and closed (red) state showing the conformational change in the presence of the calcium signal. From our collaborative paper with R. Sowdhamini of NCBS, Sunitha et al., Bioinform Biol Insights 6: 203 (2012).

RECENT PUBLICATIONS

- Margaret Sunitha S., Mercer, J.A., Spudich, J.A., and Sowdhamini R. 2012. Integrative structural modeling of the cardiac thin filament: energetics at the interface and conservation patterns reveal a spotlight on period 2 of tropomyosin. Bioinform Biol Insights.6:203-23.
- Sotelo, J.R., Canclini,L., Kun, A., Sotelo-Silveira, J.R., Xu,L., Wallrabe, L., Calliari, A., Rosso, G., Cal, K., and Mercer, J.A. 2013. Myosin-Va-dependent cell-to-cell transfer of RNA from Schwann cells to axons. PLoS ONE 8(4): e61905. doi:10.1371/journal.pone.0061905
- Sotelo, J.R., Canclini,L., Kun, A., Sotelo-Silveira, J.R., Calliari, A., Rosso, G., Cal, K., Bresque, M., DiPaolo, A., Farias, J., and Mercer, J.A. Glia-to-axon RNA transfer (review). Developmental Neurobiology, in press.

HONOURS AND AWARDS

James A. Spudich - 2012 Albert Lasker Basic Medical Research Award, 2013 Ahmed H. Zewail Award Gold Medal

INVITED TALKS

James A. Spudich

Albert Szent-Györgyi Lecture, Eötvös University, Budapest, Hungary
Plenary Lecture, European Cytoskeletal Forum, Pecs, Hungary
Many others including:American Society for Cell Biology, San Francisco

John A. Mercer

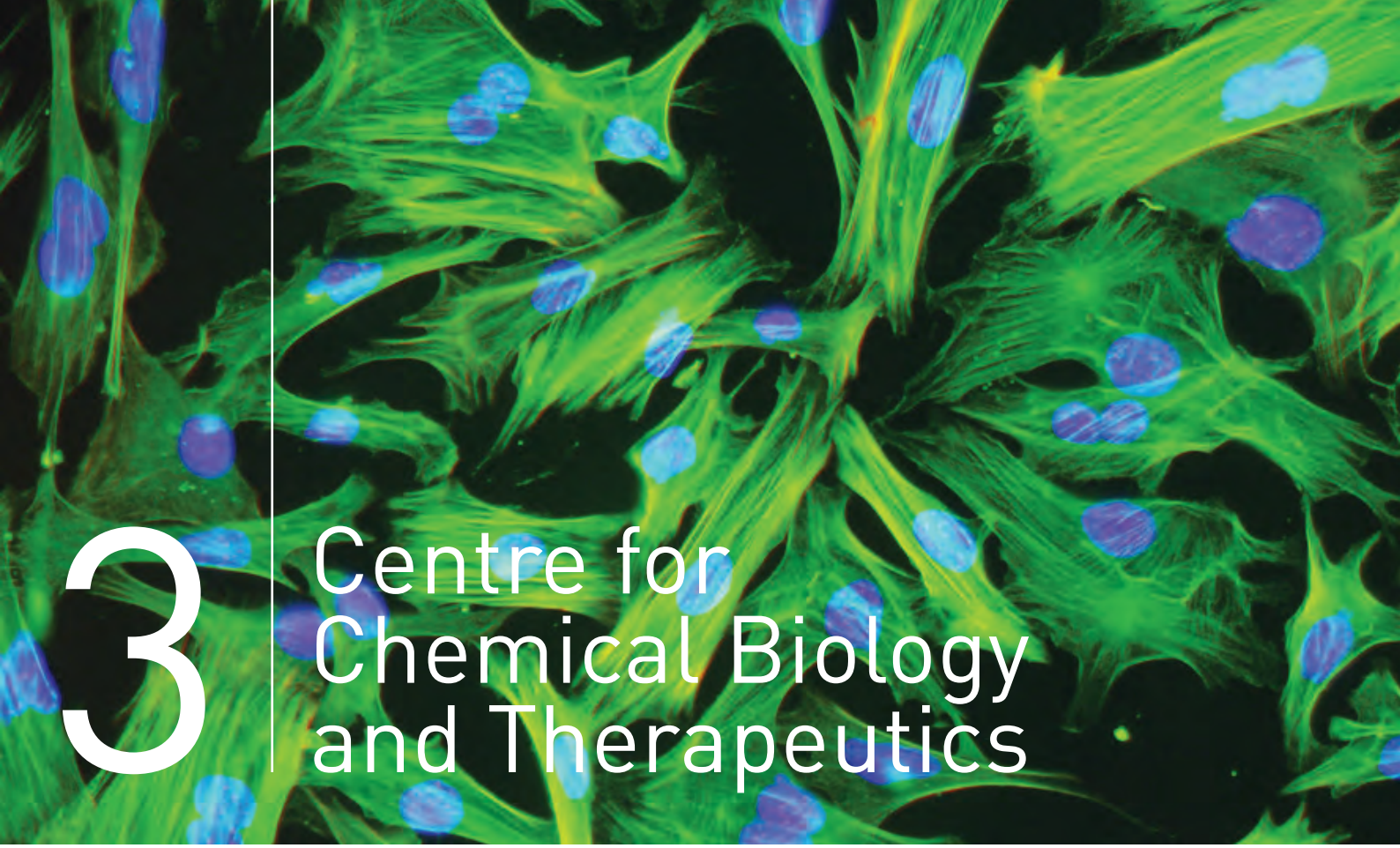
Advanced School on Axonal Transport and Neurodegenerative Disorders, Mumbai
International Conference on Cardiomyopathy Research, Chandigarh
Mechanical Manipulations and Responses at the Scale of the Cell and Beyond, Bangalore
American Society for Cell Biology, San Francisco
International Society for Transgenic Technologies, Guangzhou

TEAM MEMBERS

Tejas M. Gupte, postdoctoral fellow; Farah Haque, graduate student, NCBS; Binnu Gangadharan, graduate student, Manipal; Namita Mukundan, graduate student, NCBS; S. Margaret Sunitha, graduate student, NCBS (part-time advised by R. Sowdhamini); Kush Mehta, summer trainee, University of Pennsylvania

COLLABORATORS

R. Sowdhamini, NCBS: structural aspects of tropomyosin mutants
Leslie Leinwand, U of Colorado: humanised-mouse project
Namrata Gundiah, IISc: single-cell contractility
Narayana Hrudayalaya, Bangalore: patient samples for iPSC - cardiomyocyte
Kouichi Hasegawa, inStem: iPSC reprogramming



3 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 CCBT's 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 catalyse scientific collaborations with existing programmes, and to promote capacity building at the interface between chemistry and biology.

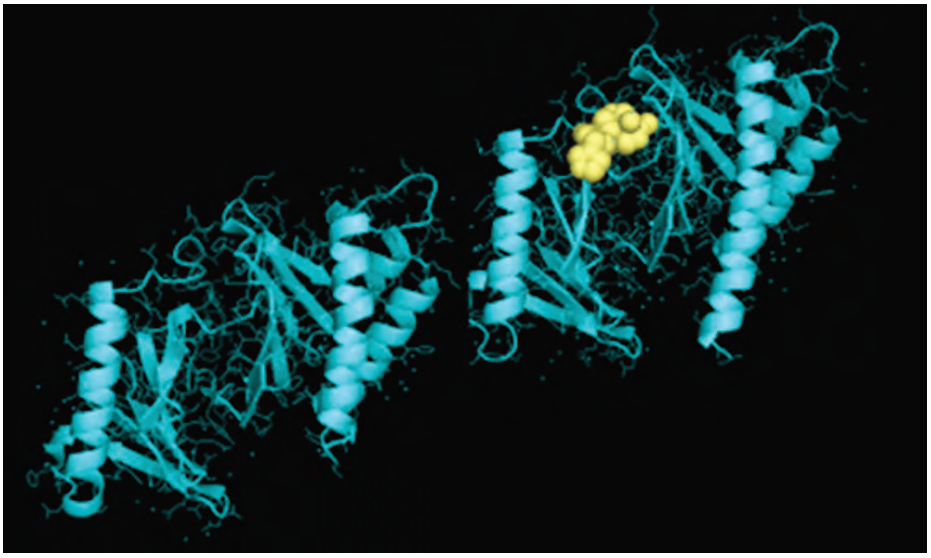
The CCBT will operate through the uniquely integrated effort of multidisciplinary project teams using common technological platforms, and not as a collection of PI-led labs pursuing independent aspirations subservient to an overall scientific theme.

CCBT theme collaborating labs work with the multidisciplinary project teams to extend research capabilities in areas of mutual interest aligned to the CCBT's scientific focus. R. 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. Yamuna Krishnan's

THEME MEMBERS

Ashok Venkitaraman
Theme Coordinator
Ashok is the Ursula Zoellner Professor of Cancer Research at the University of Cambridge, and the Director of the Medical Research Council (MRC) Cancer Unit at the Hutchison/MRC Research Centre. He is a Collaborative Science Chair at inStem and NCBS, Bangalore, and coordinates the Centre for Chemical Biology and Therapeutics there. Ashok trained in clinical medicine at the Christian Medical College, Vellore, before completing his Ph.D. at University College, London, and his post-doctoral work at the Medical Research Council's Laboratory of Molecular Biology in Cambridge. His first faculty appointment was also in the MRC Laboratory of Molecular Biology, and he remained there as a faculty member until his election as the first holder of the Zoellner Professorship.

Ashok is internationally recognised for his research on the molecular mechanisms that preserve chromosome structure and number during cell division, and how defects in these mechanisms cause human cancer. His discoveries shed light on how chromosomal instability contributes to human cancer, provide scientific foundations underpinning new approaches to therapy, and reveal new insights into the fundamental mechanisms that repair, duplicate, and segregate DNA when cells divide. Translation of these discoveries to clinical application is a major focus of Ashok's ongoing work. Ashok was elected a Fellow of the Academy of Medical Sciences, London, in 2001, and as a member of the EMBO European academy, Heidelberg, in 2004.



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.

The CCBT is funded by a direct award from the Department of Biotechnology, Government of India, as an inter-institutional collaborative centre between inStem and NCBS.

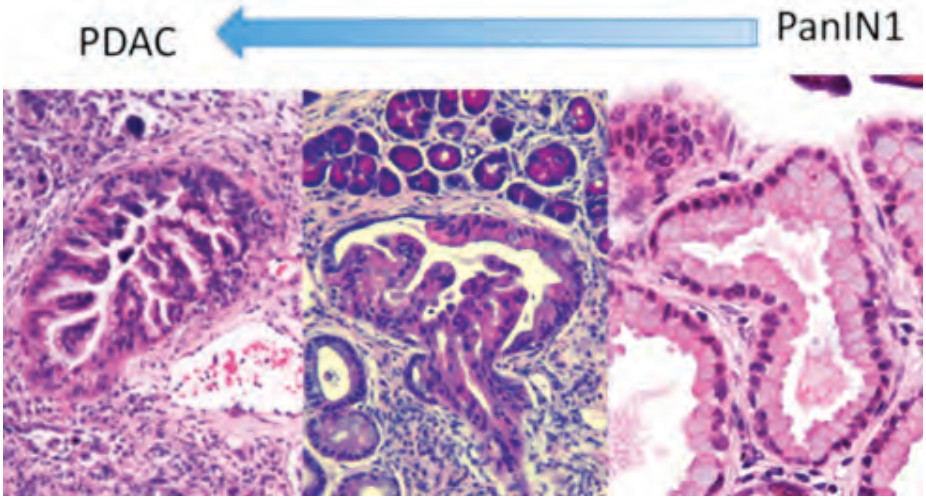
Research programme, goals, and progress:
Despite the explosive recent increase in our understanding of the genetic basis for human diseases, there has not yet been a commensurate increase in the translation of this information to the development of new medicines. Addressing this challenge is widely perceived to be a major priority for government, academia, and industry, in the light of which diverse efforts are starting to emerge in many countries.

Arguably the major problem impeding the development of new drugs is that fewer than 10% of the proteins encoded in the human genome are considered to be tractable targets for modulation using small molecules. In this regard, the emerging concept of modulating the activity of protein targets by using small molecules to alter their regulatory interactions with other proteins offers several important advantages. For instance, such an approach is expected to enhance the selectivity of small-molecule inhibitors, since regulatory interactions are less likely to be conserved between related proteins than are conserved features like active sites. Furthermore, it could considerably extend the repertoire of targets that are tractable to small-molecule drugs.

However, there are three significant and inter-related obstacles that currently preclude the routine identification of chemical leads that modulate protein-protein interactions, and their development as selective tools to enhance understanding of complex biological systems (chemical biology) or to seed the creation of new drugs. First, despite increasing interest in this problem worldwide, the scientific means and technological platforms to identify chemical leads that modulate protein-protein interactions are poorly developed. Second, there is little information concerning the potential mechanisms that could

be exploited to modulate such interactions, and the potency and selectivity of chemical leads that would be necessary to achieve a desired phenotypic effect. Third, appropriate preclinical and clinical models for the validation of therapeutic concepts underlying the potential use of protein-protein interaction inhibitors for the treatment of human diseases remain to be fully developed.

The technically challenging nature of these obstacles, and the inter-relationships between the underlying scientific questions, mean that the problem of targeting protein-protein interactions with small-molecule tools for chemical biology and to seed the development of new drugs can only be tackled in an integrated multidisciplinary centre. Platforms for the identification of chemical leads require expertise in protein biochemistry, biophysics, structural biology, computational, and synthetic chemistry, which must be integrated with somatic cell genetics, molecular cell biology and fluorescence imaging



to enable the optimisation of leads into selective chemical tools. Similarly, the development of new preclinical and clinical models for hypothesis-led translational application will require the integrated work of molecular biologists, transgenic mouse modelers and clinicians.

These considerations underpin the goal of the CCBT, which will be an integrated multidisciplinary research centre designed to pioneer new approaches to create small-molecule tools that target novel classes of targets for chemical biology and molecular therapeutics, and use them to conduct hypothesis-generating and hypothesis-driven translational research on systems of biomedical importance.

The team has moved swiftly to initiate the project. The first CCBT team leader, Muralidhara Padigaru, has joined the team from his previous position as the Head of assay development and biomarker discovery at Piramal, and has begun to recruit further administrative and scientific staff. The new laboratories are being fitted out on level 3 of the Southern Laboratory Complex (expected completion in January 2014). Research is expected to begin by the end of 2013.

Muralidhara Padigaru
Team Leader
Murali obtained his Ph.D from the Post Graduate Institute of Medical Education and Research, Chandigarh, followed by postdoctoral research at Astra Zeneca India, the US National Institutes of Health, Bethesda, and the Albert Einstein College of Medicine, New York. His major academic interest was in human genetics and genomics, where he contributed to the human genome sequencing project and to the discovery of novel disease genes implicated in bipolar disorder and narcolepsy.

COLLABORATORS
R. Sowdhamini (NCBS)
Yamuna Krishnan (NCBS)

4 Centre for Inflammation and Tissue Homeostasis

Tissue homeostasis is the functional and structural maintenance of the organs of the body. It depends on as yet not fully understood genetic, molecular, and cellular processes involved in the repair of structures and functions damaged by the normal functioning of the tissues, disease, and/or trauma. The primary barrier between these tissues and the external environment is the sheet of epithelial cells that prevent the invasion of microbes and environmental toxins from the external milieu while preventing the excessive loss of water from the body. As the primary mode of protection from physical and chemical assaults, the epithelium is often damaged and has evolved an amazing capacity to rapidly repair itself. One goal of medicine has been to harness the intrinsic self-renewing ability of damaged tissues to restore their normal structure and function. Stem cells residing in specialised niches of adult tissues are critical actors in homeostatic processes and failures in these regulatory mechanisms provoke disease and are an underlying cause of aging.

To realise the goal of regenerative medicine requires the acknowledgement and understanding of the pivotal role inflammation plays in this process. Inflammation is the first response of a tissue to damage and subsequently it plays critical roles in bringing various aspects of the healing response to a successful conclusion. Moreover, the trigger for an inflammatory response is exquisitely sensitive to disruptions in the equilibrium of the sheets of epithelial cells that form protective barriers in the body. How epithelial cells relay the information of perturbed homeostasis to initiate an inflammatory response is a crucial early event in the wound-healing programme. Likewise, the ability to link the completion of the tissue repair response to the termination of the inflammatory response is an important factor in preventing an immune response from transforming from a protective into tissue damaging agent.

THEME MEMBERS

Colin Jamora
Theme Coordinator
Associate Investigator at inStem, IFOM-inStem Research Laboratory

The Jamora lab is interested in elucidating the signaling pathways that mediate the inflammatory response, stem cell proliferation and migration, and tissue remodeling processes of the wound healing response in the skin. Investigations are also underway that probe the diseases that arise when aspects of the wound healing programme are perturbed including diabetes and tissue fibrosis.

Ramanuj DasGupta
Associate Professor at New York University School of Medicine,
Visiting Professor at inStem
Focus: The regulation of asymmetric stem cell division by Wnt signalling and its impact on tissue homeostasis.

Srikala Raghavan
Assistant Investigator
She is interested in understanding the role of integrins in regulating epidermal homeostasis; mechanism(s) by which altered tissue homeostasis induces an inflammatory response; role of small RNAs in epithelial stem cells.

This occurs because inflammation involves both innate and adaptive immune elements, the endothelium, and the complement and coagulation cascades. When the brakes on the inflammatory response are impaired, prevalent chronic inflammatory diseases such as rheumatoid arthritis, diabetes, atherosclerosis, psoriasis, and fibrosis can arise. Consequently, a thorough understanding of the regulation of the inflammatory response would engender new clinical insights into the treatment of many prevalent diseases when this process goes awry.

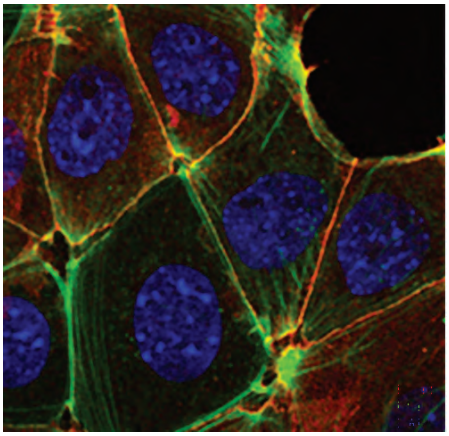
Despite enormous progress in studying the immune system, a holistic understanding of the inflammatory response is not yet in grasp. In addition to their classical role of mounting a counterinsurgency to ward off invaders crossing the breached epithelial barrier, new paradigms on the role of immune-derived cytokines on various cell types including stem cells are emerging. Anectodal evidence is now appearing in the literature that inflammatory cells can regulate cell proliferation, differentiation, cell-fate specification, and tissue migration, invasion, remodeling of multiple cell types. For instance, interferon-gamma (IFNγ) and tumor necrosis-α factor a (TNFα) have recently been implicated in guiding cell fate specification of hematopoietic stem cells. These non-immune functions of inflammatory cytokines can therefore have a profound impact on tissue regeneration. Thus, a systemic understanding of the combinatorial effects of inflammatory cytokines upon the varied cells within a tissue remains a daunting challenge that needs to be addressed. Filling this gap in our knowledge will overcome a major roadblock that is hindering the development of effective therapies in regenerative medicine. The major goals of this theme are two fold:

- The study of immune regulation at barrier surfaces
- The regulation of the numerous cells that mediate tissue regeneration and repair

Progress and Ongoing work

The group leaders guiding the research activities of CITH include: Srikala Raghavan, Shravanti Rampalli-Deshpande, Colin Jamora, and Ramanuj DasGupta.

A multi-pronged approach is brought to bear by the different groups to understand how alterations in epidermal homeostasis can elicit an inflammatory response and how stem/progenitor cells are regulated in order to carry out the careful and complex programme of tissue repair. Inherent in these studies is a strong emphasis on basic cell and developmental biology studies borne from the need to understand the fundamental blueprint of tissue development and homeostasis. To a large extent, tissue repair is the process of tissue development with an added layer of an inflammatory response that not only wards off infection, but is increasingly being recognised for the ability to influence the cellular decisions of the cells within the inflammatory milieu. Consequently, how cellular behaviour is impacted by inflammatory cytokines is another area of interest pursued by CITH members.



Programme 1.
The study of immune regulation at barrier surfaces

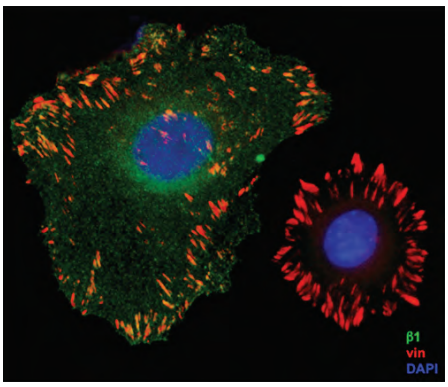
A major question in the field of inflammation is how do you initiate an immune response and terminate it after a defined period of time. Compromising either the start or end of an inflammatory response can lead to debilitating states such as infection and sepsis or, at the other extreme, chronic inflammatory diseases. Both subtle and dramatic alterations in the sheet of epithelial cells that comprise the barrier of various organs in the body are known to elicit an inflammatory response. Though this phenomenon is well-documented, a mechanistic understanding of how epithelial homeostasis governs an inflammatory reaction is still far from reach.

The assembled team is in a distinctive position to tackle this problem owing to their long-standing interest and expertise in epidermal homeostasis combined with innovative mouse models generated in their labs with which to address this problem. The epidermis in particular is an outstanding platform for investigating tissue homeostasis given that it is one of the few tissues that constantly regenerates throughout the lifetime of the animal. Moreover, due to its protective function as a barrier from the external environment, the epidermis is constantly damaged and must mount a wound-healing programme to swiftly restore tissue architecture and function. Interestingly, this tissue repair processes is akin to the developmental process of tissue morphogenesis with the added complexity of inflammatory cell signaling layered upon it.

The laboratory of Srikala Raghavan has one of the few viable mouse models in which $\beta 1$ integrin has been conditionally ablated from the epithelial compartment of the skin. The primary interest of the Raghavan lab is to elucidate how integrins and their associated proteins maintain epithelial homeostasis during development and disease. The integrin KO mouse thus offers an excellent model system to address the nexus between tissue organisation and signals mediating an immune response that may be involved in the process of healing without leaving scars in embryos. The work on the crosstalk between epidermal homeostasis and inflammation is further expanded in the laboratory of Colin Jamora, who has generated and characterised the skin of mice lacking caspase-8 expression specifically in the epidermis. Analysis of this caspase-8 conditional knockout mouse uncovered a novel function for caspase-8, which has classically been studied in the context of its role in mediating apoptosis. Interestingly, the loss of epidermal caspase-8 recapitulates many of the cardinal features of a wound-healing response in the absence of any damage to the skin.

Shravanti Rampalli-Deshpande
Assistant Investigator
She is addressing the areas of basic human stem cell biology to identity how cell fate is acquired and maintained. For this the team studies the role of chromatin modifying enzymes in cellular differentiation/ de-differentiation and characterises the signaling pathways that intersect with chromatin modulators to govern cell fate decisions.

Current research in the Jamora lab is focused on testing the hypothesis that epidermal caspase-8 may act as a sensor to monitor epidermal integrity. One question that arises from this line of inquiry is the mechanism by which caspase-8 gene expression is normally reduced in epidermal cells at the wound site. Preliminary evidence suggests that the downregulation of this gene is partly due to the methylation of the caspase-8 locus (a similar phenomenon which has been documented in various cancers). This study will include the collaboration of Shravanti Rampalli-Deshpande who has the expertise in understanding the epigenetic regulation of somatic and stem-cell plasticity. In addition, how the downregulation of caspase-8 in response to trauma or damage elicits the inflammatory, proliferative, and remodeling phases of the wound-healing programme through extensive intercellular interactions is a major effort in the lab. Interestingly, diabetics with an impaired wound-healing response also exhibit a failure to lower caspase-8 levels following injury. As such, the Jamora lab is also using mouse models of diabetes to complement their own studies on the regulation of caspase-8 gene expression and the downstream signaling effects reducing this protein has on the initiation and progression of the wound-healing response. In summary, major research programmes of the Raghavan and Jamora labs will provide important insights into how alterations in tissue organisation are sensed and translated into a cutaneous inflammatory response. Given the fact that both labs have published the phenotypic characterisation of both mouse models, the groups are now at a unique position to identify important components linking the epithelia to the immune system.



Programme 2.
The regulation of the numerous cells that mediate tissue regeneration and repair

In response to a wound in the skin, hair follicle stem cells that are normally dedicated for follicle regeneration are redirected to the epidermis to contribute to the rapid repair of this tissue. Raghavan and DasGupta sequenced the transcriptome of the murine hair follicular bulge stem cells that are important contributors to epidermal repair, using next-gen-sequencing (NGS), and compared it to that of transit-amplifying (TA) and differentiated cells. These preliminary findings provide the research group with a unique opportunity to test the function of miRNAs and novel tsRNAs in the regulation of homeostasis in murine epidermal/follicular stem cells. Moreover, they are now equipped to assess how an inflammatory microenvironment impinges on stem cell behaviour at the level of RNA regulation. This area is of specific importance as there is a growing realisation that inflammatory signals can affect multiple behaviours of stem cells in a variety of tissues though the mechanism by which this is accomplished is still virgin territory. This work dovetails nicely with the studies in the Jamora lab focused on the regulation of epithelial stem cell behaviour during wound-healing using the conditional knockout of caspase-8 as a model system. A sample question in this regard is the mechanisms controlling the

chemotaxis of epithelial stem cells from various niches in the skin to the site of tissue damage. A thorough characterisation of the gene expression profiles of stem cells vs. transit amplifying cells vs. differentiated cells will provide the necessary framework to understand how changes in stem cell behaviour are elicited at the genetic level.

Though hair follicle stem cells and other epithelial skin stem cells are major components of the wound-healing programme, the process of tissue repair is nevertheless reliant on a constellation of other cell types. Of particular interest for this group are the dermal fibroblasts that play an essential (though often overlooked) role in wound healing. One of the observed functions of these dermal fibroblasts in wound healing is their predicted potential to differentiate into multiple cell types within a tissue. Recent literature results raise the possibility that under-defined conditions such as inflammation, the fibroblasts can be forced to differentiate towards specified lineages. While this is an intriguing hypothesis, several key issues remain largely unexplored. For example, does every fibroblast cell exhibit developmental plasticity? Furthermore, what is the molecular and epigenetic basis of this observed plasticity? Interestingly, these epigenetic contributions to determine dermal fibroblast fate can be strongly influenced by the particular microenvironment in which the cell resides.

Rampalli's laboratory will investigate the molecular and epigenetic differences in the population of dermal fibroblast during the process of wound healing. Research in her lab will be focused on understanding the interplay between epidermal stem cells and fibroblasts in response to TGF-β, an extracellular signal known to play an important role in wound healing, and test the impact of epigenetic regulators on fibroblast plasticity. Rampalli has already performed a microarray profile from adult, fetal, and neonatal fibroblasts and generated fibroblasts that are depleted of methylases and demethylase. Currently, her lab is performing RNA sequence analysis of these mutant fibroblasts. The sequencing data from all the sources will be compared with special emphasis on genes involved in epidermal stem cell interaction and wound repair. Rampalli plans to employ *in vitro* wound-healing assays and mouse models in collaboration with Jamora for the proposed project. The *in vivo* relevance of these findings in relation to the ability of dermal fibroblasts to repopulate various structures within the skin will be done in collaboration with Raghavan, who has the expertise in grafting cells onto the skin of mice to determine their ability to differentiate among various lineages. Moreover, her lab will extend the studies to understand differential effect of epigenetic regulator depletion on fibroblast plasticity in de-differentiation or trans-differentiation of other somatic cell types through comparative studies using previously reprogrammed epidermal stem cells such as keratinocytes from the skin. As such, the NGS profile of various epithelial stem cells obtained by Raghavan and DasGupta will be an important resource in these comparative studies. Validation and filtering of the NGS sequencing data will take 6-8 months of work following the placement and training of appropriate personnel. Analysis of the contribution of selected genes will first be tested in an *in vitro* scratch wound assay followed by the *in vivo* analysis of wound healing in the skin of mice.

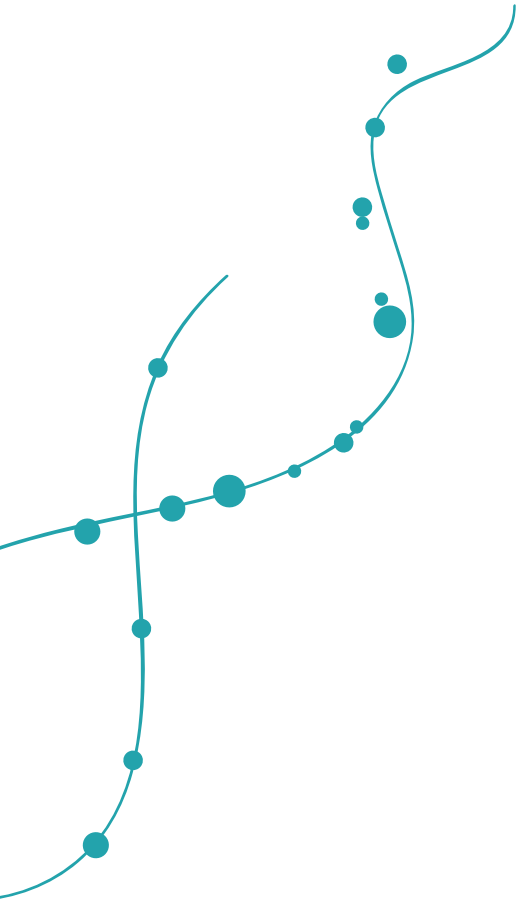
HONOURS AND AWARDS

Jamora 2012 American Society for Cell Biology MAC Travel Award
2012 Keystone Symposium Early Career Investigator Travel Award

Dasgupta 2010-2013 March of Dimes Research Grant, USA
2011-2015 American Cancer Society, Research Scholar Grant, USA
2012-2014 NYC BioAccelerate Prize, New York, NY, USA

Raghavan 2012-2016 Research Scholar Grant, American Cancer Society, USA
2012 Daniel M. Laskin award for most outstanding article published in JOMS.

Rampalli 2011-2015 Wellcome Trust/DBT India Alliance Intermediate Fellowship Award



INVITED TALKS

Jamora

- Centre for Stem Cell Research-inStem Joint Research Talks. Bangalore, India 2012.
- IFOM-Kyoto University Joint Symposium on Mechanisms of Cell Transformation and Metastasis. Milan, Italy 2012.
- International Conference on Stem Cells and Regenerative Medicine. London, England 2012.
- Imperial College London (Centre for Inflammation and Cell Signaling). London, England 2012.
- Scleroderma Research Foundation Annual Workshop. San Francisco, CA 2012.
- Case Western Reserve University (Department of Biology) 2012.
- University of Illinois, Chicago (Department of Pharmacology) 2012.
- California State University, Northridge (Department of Biology), 2012.
- Sanford Children's Health Research Centre, Sioux Falls, South Dakota, 2012.

DasGupta

- Feb 2012 Speaker, Stem Cell Retreat, NYU School of Medicine, NY.
- April 2012 Invited speaker, NYU-AD, Abu Dhabi, UAE.
- May 2012 Invited speaker, HFSP stem cell meeting, Boston, MA.
- July 2012 Invited speaker, University College, London, UK.
- Oct 2012 Invited speaker, Duke-National University of Singapore, Singapore.
- Oct 2012 Invited speaker, IMCB, Biopolis, Singapore.
- Dec 2012 Invited speaker, Centre for Stem cell Research, CMC Vellore, India.
- Jan 2012 Invited speaker, GIS, Biopolis, Singapore.
- Feb 2013 Invited speaker, NYU-Abu Dhabi, Stem Cell Meeting, Abu Dhabi, UAE.
- March 2013 Invited speaker, Dept. of Molecular Biology & Biochemistry, SFU, Canada.

MEETINGS ATTENDED

Jamora

- Unilever-NCBS Symposium. Bangalore, India 2013. "Wound healing and related diseases."
- IFOM-Kyoto University Joint Symposium on Mechanisms of Cell

Transformation and Metastasis. Milan, Italy 2012. "Mechanism by which epidermal stem cells generate a tumor promoting microenvironment in the skin."

- Scleroderma Research Foundation Annual Workshop. San Francisco, CA 2012. "Using the Snail transgenic mouse to understand scleroderma fibrogenesis."

Raghavan

- International Conference on Stem Cells and Regenerative Medicine. London, England 2012. "Regulating Epithelial Stem Cell Behaviour in a wound microenvironment."

Rampalli

- Invited speaker for the talk entitled " Epigenetic Regulation of Pluripotency and Differentiation in human Stem Cells" at IFOM-IEO-NCBS meeting. NCBS Bangalore, 26 April 2012.
- Invited speaker for the talk entitled " Epigenetic Regulation of Pluripotency and Differentiation in human Stem Cells" at the International Conference on Stem Cells and Regenerative Medicine. University College London, July 11 2012.

RECENT PUBLICATIONS

- Jamora C. (in press).Mechanisms of Wound Repair. Stem Cells. (Federico Calegari and Claudia Waskow, editors). Science Publishers Edenbridge Ltd.

TEAM MEMBERS

Jamora Lab:
Postdoctoral Fellows – Brijesh Ajjappala, Subhasri Ghosh (effective Nov. 2013)
PhD students (NCBS graduate programme) – Sunny Kataria, Tanay Bhatt
Junior Research Fellows – Syed Abrar Rizvi (CSIR), Surya Prakash Rao, Edries Yousaf Hajam
Interns – Federica Carlomagno (visiting M.Sc. student from IFOM)

DasGupta Lab:
Postdoctoral Fellow – Mallika Ramakrishnan
Junior Research Fellow - Pratyusha Pavanram

Raghavan Lab:
Junior Research Fellows- Ambika Kurbet, Anupama Ashok, N. Kavana, Samarth Hegde

Rampalli Lab:
Postdoctoral Fellow - Alhad Ashok Ketkar
Junior Research Fellows– Radhika Apte, Narendra Dhele, A. Rashika Rao, Ganeshkumar Arumugam

COLLABORATORS

Jamora:

- Shyni Varghese (UC San Diego, Department of Bioengineering) to understand the mechanochemical cues contributing to the formation of scar tissue following tissue injury
- Emmanuel Theodorakis (UC San Diego, Department of Chemistry and Biochemistry) to elucidate the mechanism by which synthetic small molecules inhibit cell migration and invasion

DasGupta:

- Roel Nusse (Stanford University, School of Medicine) in a collaborative project funded by the California Institute for Regenerative Medicine (CIRM) and the Department of Biotechnology (DBT) to understand the role of Wnt signaling in asymmetric division of epithelial stem cells

Raghavan:

- David Calderwood (Yale University, Department of Pharmacology) to study the mechanism of kindlin signaling in mouse keratinocytes

5 Technologies for the Advancement of Science

The TAS team aims to develop technologies, use technologies as the driver for science, and act as a catalyst for the advancement of science at inStem, at the team's campus, and beyond.

The most innovative aspect of this team is the coming together of a group of individuals with vastly different expertise from mouse genetics to x-ray crystallography and vastly different research interests from organogenesis to electron flow in biology. The idea of working together as a team with weekly team meetings, retreats, students and post-docs, core facilities, and a common aspiration to be at the cutting-edge of science and technology is unique. Often multi-disciplinary team-driven activities are top-down. At TAS experimentation is conducted in a bottom-up approach. Top-down approaches more often lead to incremental improvements, have in-built constraints, and are not always conducive to innovation. Bottom-up approaches are high-risk, but have the ability to make quantum leaps. By increasing the chance of collision of seemingly different scientific and technological interests, we are exponentially increasing the probability of producing disruptive science and technology. As any good biochemist will know, a linear reduction in activation energy leads to an exponential increase in the rate of catalysis, which depicts TAS' activities at inStem.

The activities can be divided into three broad areas.
1. Providing core support for research in stem cell biology and regenerative medicine. This includes the stem cell facility, the chemistry core, and the mouse genetics facility.

2. Establishment and development of technologies that are not core facilities, but important for advancing the science of the drivers as well as the science on

THEME MEMBERS

S. Ramaswamy
Senior Professor
Dean at inStem; CEO of C-CAMP
He has experience in technology and methodology development, setting up and managing facilities, working with and leading teams. His scientific interests are in the broad area of biophysical chemistry.

Jeff Abramson
Professor, Department of Physiology
UCLA School of Medicine,
Collaborative Science Chair
He is today recognised as one of the top membrane protein structure-function scientists in the world. His primary interests are in sugar transport and its link to life style diseases.

Dasaradhi Palakodeti
Senior Fellow
Wellcome/DBT Intermediate Research Fellow
He is a developmental biologist, with interest in studying pluripotency in model systems like planaria and hydra. He has significant expertise in RNA biology and Next Generation Sequencing tools.

Praveen Vemula
Senior Fellow
Ramalingaswami Fellow
He is a chemist turned nano-materials and drug delivery expert. Other than being a scientist, he was also the Kauffman entrepreneurial fellow at Harvard. He is also involved in setting up companies. His expertise in translation and focus on application will make sure the team's work has relevance to more than mere publications.

campus and outside. These vary in scope - from setting up the technologies on campus, developing new approaches, and integrating technologies to provide innovative solutions to completely developing new technologies and tools.

3. Scientific programmes. As part of the TAS team, there must also be projects that are purely science driven to keep the creative energies of the team at a high level and provide the imaginative edge to their technology development. These projects, other than having scientific merit, also bring together technologies, tools, ideas, and people.

Collaborative elements

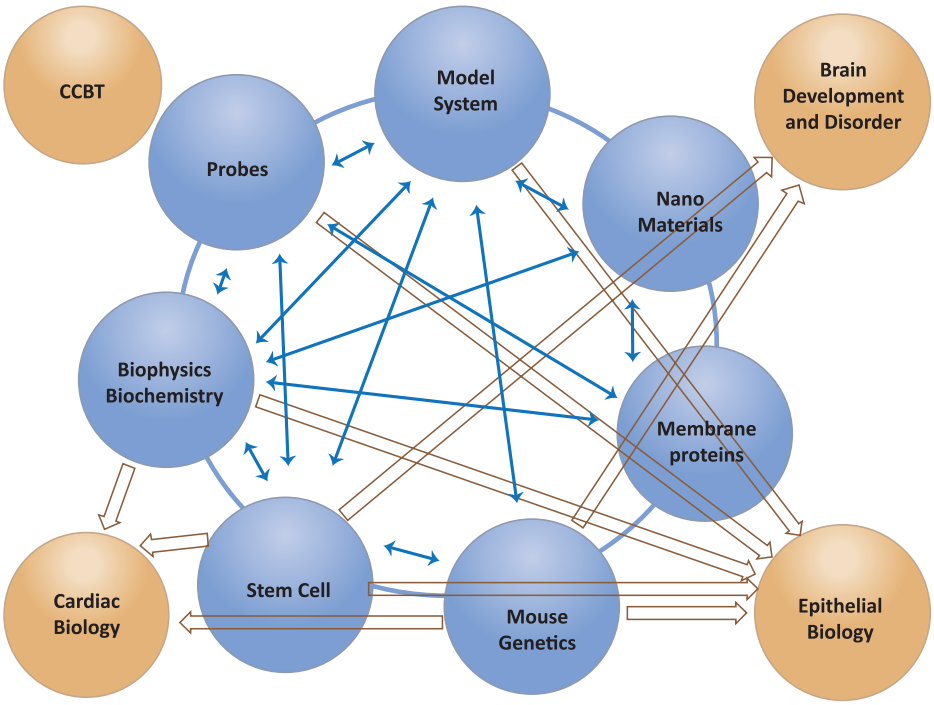


Figure 1: Describes the collaboration between different PIs within the team (blue circles and in blue arrows) and with different teams (tan circles and tan arrows) at inStem.

THEME MEMBERS

The members of the team are the reason for its success. While extremely prominent, it is a very small fraction of scientists who have achieved significant heights in basic science and have started companies. It is rare that one finds people with expertise in developmental biology, nano-materials, chemical biology, and technology development. Prof. Norio Nakatsuji, visiting professor at inStem and mentor of this team, is one such person. His advice will be critical to the team's overall goals and direction.

The Theme Member profiles are given on the outer columns.

The TAS team has been very fortunate in attracting outstanding scientists. These include Sai Sudha, Yashoda Ghanekar, Aude Conscience, Vinod Nayak, Subhashini Sadasivam among others. The team also interfaces with C-CAMP which provides access to cutting-edge technology platforms.

In addition, the TAS team includes an inStem Associate (Prof. Anil Prabhakar, Electrical Engineering, IIT-Madras) and a sabbatical visitor (Prof. Balaji Rao, Chemical Engineering Department, North Carolina State University).

RESEARCH HIGHLIGHTS

Role of glycans in regeneration: In order to elucidate the role of glycans in regeneration, the TAS team has carried out whole cell gylcomics and identified the entire glycan profile of the model organism Hydra magnipapillata. The results revealed a so far unidentified polyfucosylated LacdiNAc structure and tri-HexNAc antenna structure. The receptor to this sugar was identified as CLECT-2. Disruption of the sugar epitope resulted in shutdown of the regeneration machinery demonstrating for the first time at the molecular level the connection between cell-surface glycans and regeneration.

Sahadevan S. et al. ACS Chemical Biology 2013

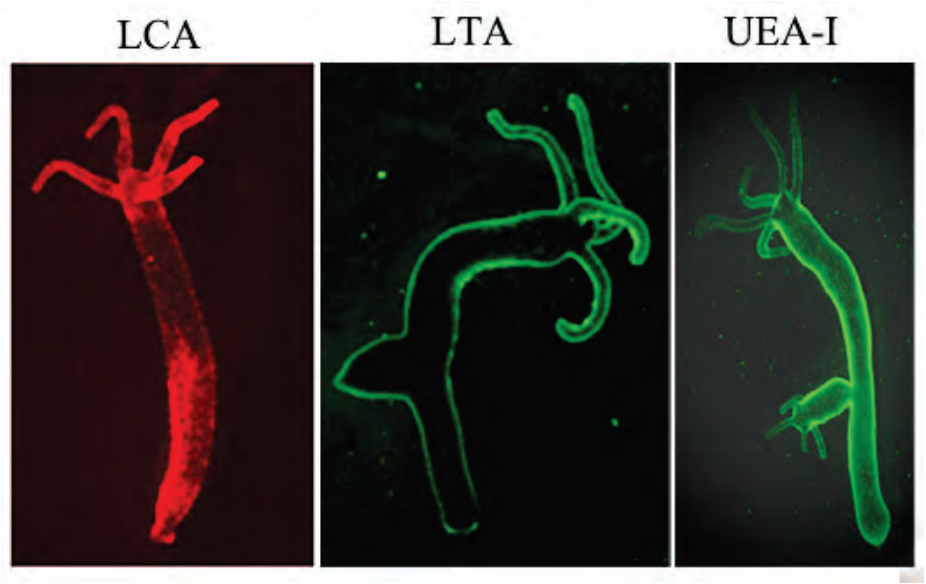


Figure 1: Fluorescent microscopy images of Hydra magnipapillata binding to LCA-Rho, LTAFITC, and UEA-I FITC.

Identification of novel microRNAs and new class of smallRNAs in regenerating Hydra magnipapillata: Hydra is a diploblastic freshwater-living Cnidarian. Hydra has been extensively used to study stem cell function and regeneration due to their robust regeneration capacity. Though several genes and pathways essential for Hydra regeneration was identified, but not much is known about the small RNAs that regulate regeneration in Hydra. Here, deep sequencing of the head regenerating Hydra at various time points was conducted to identify the small RNA that are enriched in the regenerating tissue at various time points. Extensive computational analysis of deep sequencing data identified several classes of small RNA such as miRNAs (microRNAs), piRNAs (piwiRNAs), and endosiRNAs in Hydra. We have identified 126 miRNA loci; 123 of these miRNAs are unique to hydra. Less than 50% are conserved across two different strains of Hydra vulgaris tested in this study, indicating a highly diverse nature of hydra miRNAs in contrast to bilaterian miRNAs. siRNAs derived from precursors with perfect stem-loop

Akash Gulyani
Assistant Investigator
His research interests are at the interface of chemistry, biology, and imaging, has already set up several cross-collaborations on and off campus. He will also host the TAS team's first visiting scientist on sabbatical.

Kouichi Hasegawa
Joint faculty between inStem-ICEMS, Kyoto
His interests are in the area of stem cell pluriopotency. Prior to joining inStem-ICEMS, he was a faculty member at the University of Southern California.

Ramkumar Sambasivan
Senior Fellow
Ramalingaswami Fellow
He is a developmental biologist. He has demonstrated expertise in mouse genetics and the ability to make transgenic mice, and is helping set up the transgenic mouse facility. He intends to study gene regulatory networks in vertebrate evolution and development - a problem usually tackled by large labs with large resources. His larger than life scientific ambitions will drive him to create an outstanding mouse SPF facility on campus.

Kenichi Suzuki
Associate Investigator at inStem in collaboration with iCeMs, Kyoto
His lab focus is single-molecule study of interactions among cell membrane components. His group is trying to understand raft signaling in the context of a) Interactions among many signaling molecules bound to actin filaments and rafts b) Protein-protein interactions and lipid involved interactions.

structure that arise from inverted repeats were also identified. piRNAs were the most abundant small RNAs in hydra, mapping to transposable elements, the annotated transcriptome, and unique non-coding regions on the genome. piRNAs that map to transposable elements and the annotated transcriptome display a ping-pong signature. Further, several miRNAs and piRNAs were identified whose expression is regulated during hydra head regeneration. The study defines different classes of small RNAs in this cnidarian model system which may play a role in orchestrating gene expression essential for hydra regeneration (Srikar et al. 2013).

Structure and function of membrane proteins: Membrane transport proteins are responsible for many critical biological functions including governing energy transduction, modifying ion concentrations, and actively importing metabolites into the cell. Membrane proteins represent ~30% of all proteins in each of the sequenced genomes and are targets for over 60% of all marketed drugs. The Abramson lab has two main foci: development of innovative tools and approaches for determining 3D structures of membrane proteins and employing these and other biochemical/biophysical tools to determine the biological basis for membrane transport.

Recent advances in the lab has determined Voltage Dependent Anion Channel in complex with ATP (Manuscript in preparation) and functional dynamics of the sodium glucose transporter using wide angle x-ray scattering and EPR. Some recent publications are listed below.

RESEARCH TEAMS

Ramaswamy S (TAS Theme Coordinator)

Research area: Protein biochemistry and structural biology, glycobiology, stem cell biology and regeneration in Hydra and Planaria.

Research description: TAS, laboratory works with model organisms, Hydra and Planaria, to study fundamental questions in stem cell biology and regeneration. Hydra is a fresh-water cnidarian capable of regenerating its entire body from a small part of the body column or from dissociated cells. This extraordinary ability renders it almost 'immortal', making it a great model system to study aging, stemness, and regeneration. Planaria is also capable of regeneration from small pieces of tissue. A small niche of specialised stem cells, called the neoblasts are pluripotent and clonogenic. Together, Hydra and Planaria are tractable and facile model systems to understand the fundamental basis of stemness and regeneration.

We primarily use cell biology techniques in studying Hydra and Planaria regeneration. The focus is on:

- Understanding redox regulation by the transcription factor, FOXO
- The structure and function of the enzymes involved in reactive oxygen scavenging
- Characterising the glycome of cell surface proteins and understanding the role of cell surface proteo-glycans in regeneration

- Studying the role and lipid profile during stages of regeneration – from neoblasts to differentiated cells and in the differentiated cells
- A new programme is underway to study the structure and function of membrane proteins – especially sugar transporters - in eukaryotes
- Several bacteria evade host's inflammatory response by coating themselves with host-sugars. We are studying the role of these scavenged sugars coated on the cell surface in biofilm formation especially in the oral mucosa

The laboratory's philosophy of encouraging curiosity-driven research has spawned a number of projects in the lab that are diverse in nature and approach.

LAB MEMBERS:
Scientists:
P. Sai Sudha - Scientist D
Yashoda Ghanekar - Career Development Fellow
Subhashini Sadashivam - Fellow

Postdoctoral Fellows:
Sathya Srinivasachari - Wellcome Trust Early Career Fellow
Arun Kumar - Postdoctoral Fellow
Nainesh Katagihallimath - Postdoctoral Fellow

Lab Staff:
Thanuja Gangisetty - Project Assistant
Wendie Ann Dockstader - Lab Manager

PhD Students:
Swagatha Ghosh
Arunabha Sarkar

Junior Research Fellows:
Lavanyaa M. - JRF
Niraimathi Govindasamy - JRF
Amrutha K.
Amrendra Mishra
Subhadra Dalwani
Srikar Krishna

RECENT PUBLICATIONS:

- Sahadevan S, Antonopoulos A, Haslam SM, Dell A, Ramaswamy S, Babu P. Unique, polyfucosylated glycan-receptor interactions are essential for regeneration of Hydra magnipapillata. ACS Chemical Biology 2013, in press.
- Plapp BV, Ramaswamy S. Atomic-resolution structures of Horse liver alcohol dehydrogenase with NAD+ and fluoroalcohols define strained Michaelis complexes. Biochemistry 2012, 51 (19), 4035-4048.
- Ver Heaul AM, Fowler CA, Ramaswamy S, Piper RC. "Ubiquitin regulates caspase recruitment domain-mediated signaling by nucleotide-binding oligomerization domain-containing proteins NOD1 and NOD2." Journal of Biological Chemistry 2013, 288(10), 6890-6902.

- One Atom Makes All the Difference. S Ramaswamy, (2011) Science 334 (6058), 914-915.

Praveen Kumar Vemula

Research Area: Vemula's lab primarily focuses on translational research. His group research interests include organic/bio-based synthesis of novel biomaterials, self-assembled nano/micro-materials and developing next-generation biomaterials to solve challenging unmet clinical/biomedical needs.

Research Description: Protection from degradation and enhancement of intracellular delivery of a wide range of nucleic acids (DNA/RNA) based therapeutics is still a huge unmet need. He aims to develop platform approaches based on non-viral self-assembled biomaterials for an efficient delivery of chemical/biological drugs and nucleic acids. Self-assembly of amphiphiles is a powerful tool to generate a wide range of biomaterials such as micelles, reverse-micelles, liposomes, bilayers, nano/micro particles, and hydrogels that could be utilized to develop a new armamentarium of biomaterials to the nucleic acid and drug delivery. Utilising organic synthesis, the team will develop multiple libraries of small molecular lipids/amphiphiles by introducing systematic structural variations to i) reduce toxicity, ii) modulate their interaction with biomolecules to form complexes, iii) enhance stability and iv) facilitate an efficient delivery. Matured technologies will be utilised for transfection of transgenes to develop transgenic planaria/hydra, delivery of microRNAs for skin therapy and wound healing, and delivery of nucleic acid vaccines.

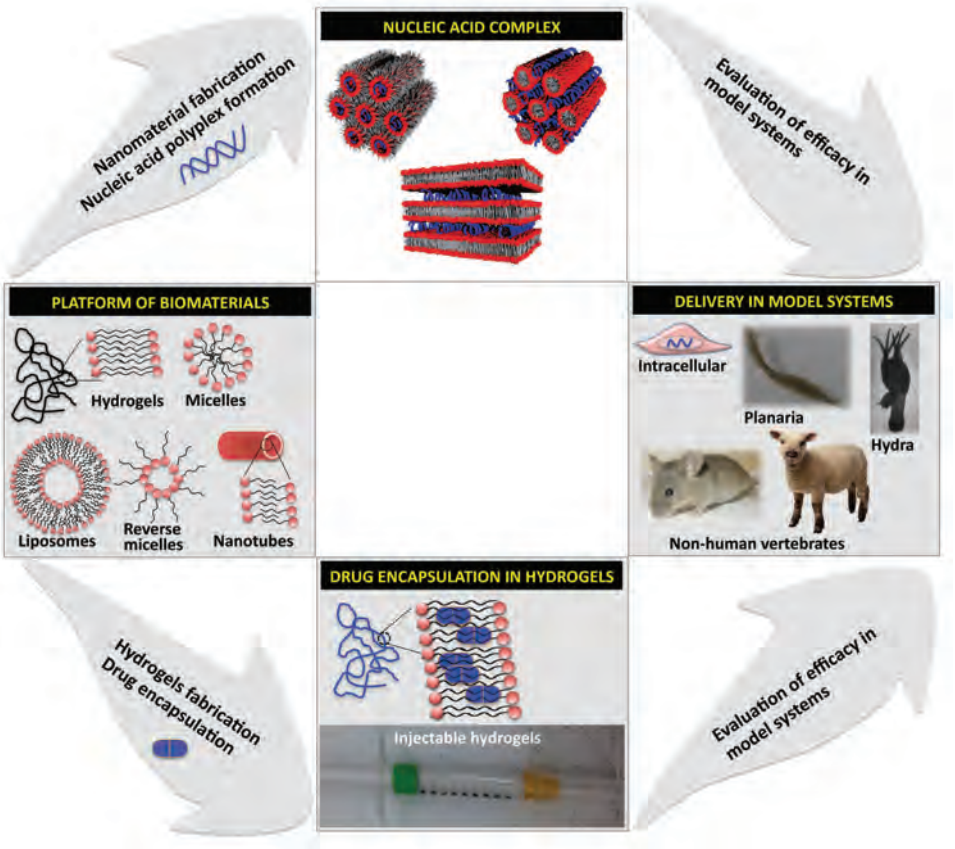


Figure 1: Schematic of developing next-generation self-assembled nanomaterials to deliver drugs and biomolecules (nucleic acids).
1) Synthesis of multiple libraries of lipids and amphiphiles, 2) fabrication of nanomaterials and encapsulation of drugs or biomolecules, and 3) evaluation of their efficacy in model systems.

LAB MEMBERS:
Debjit Dutta – Postdoctoral Fellow
Ashish Dhayani – Junior Research Fellow
Ketan Thorat – Junior Research Fellow

COLLABORATORS:
- Robert Rieben (University of Bern, Switzerland) – Therapeutics for organ transplantations
- Jeffrey Karp (Harvard Medical School, USA)- Therapeutics for organ transplantations
- Leslie Yeo (RMIT University, Australia) – Inhalable DNA-vaccines
- David Piedrafita (Monash University, Australia) – Inhalable DNA-vaccines
- Sahul Hameed (C. Abdul Hakeem College, Vellore) – Delivery of immunostimulators in marine organisms

RECENT PUBLICATIONS:

Balachandran VS, Jadhav SR, Vemula PK,* John G.* "Recent advances in cardanol chemistry in a nutshell: from nut to nanomaterials." Chemical Society Reviews 2013, 42, 427-438. (Cover Page article) (*co-corresponding authors)

Vemula PK,* Wiradharma N, Ankrum JA, Miranda OR, John G, Karp JM.* "Prodrugs as self-assembeld hydrogels: a new paradigm for biomaterials." Current Opinion in Biotechnology 2013 (in press). (*co-corresponding authors)

HONOURS AND AWARDS

Ramalingswami Re-entry fellowship, Department of Biotechnology, Government of India (2012-2017)

INVITED TALKS

- Title: "Design of biobased nanomaterials: A new paradigm in developing therapeutics."
  Venue: Inviated talk at Indian Institute of Technology, Gandhinagar, Symposium on 'Drug Discovery and Development'. May 25, 2013
- Title: "Nano-therapeutics: Biobased nanomaterials to prevent skin disorders"
  Venue: Invited talk at Indo-USA international conference on NanoBio-2013, Tiruchirappally. June 27-29, 2013
- Title: "A new paradigm in nanotherapeutics: Next-generation nanomaterials to prevent skin disorders."
  Venue: Invited talk at Sri Venkateshwara University, Tirupati. July 12, 2013
- Title: "Design of biobased nanomaterials: A new paradigm in developing therapeutics."
  Venue: Invited talk at 1st International Conference on Emerging Trends in Bioengineering, at SASTRA University, Thanjavur. August 9-10, 2013

- Title: "Inflammation-responsive on-demand drug delivery: A new paradigm in Vascularised Composite Allotransplantation."
  Venue: Invited talk at Symposium on Vascularised Composite Allotransplantation, at Inselspital, University of Bern, Bern, Switzerland
  Date: September 2, 2013

SEMINARS HOSTED:

Praveen Vemula recently hosted a campus visit for faculty, staff, and multidisciplinary students (15, Self Engineering Leadership Fellowship receivers) from University of Kansas, USA. Half-day visit to the campus on August 21, 2013 enabled interactions with students and faculty from this campus. This engagement may mature into an exchange students internship programme.

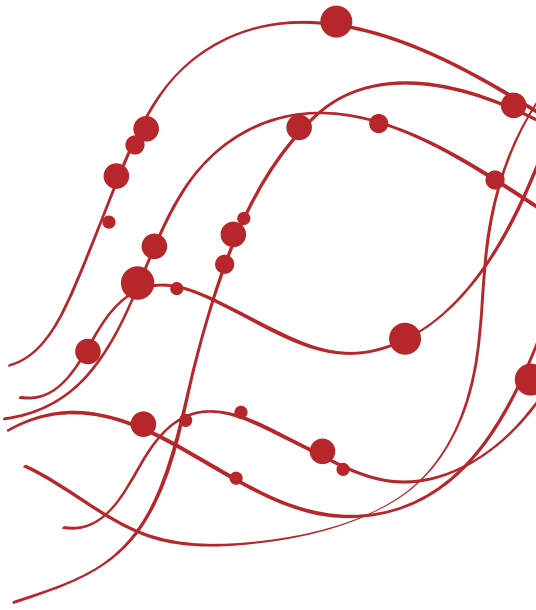
Ramkumar Sambasivan

Research Area: Developmental biology.

Research Description: The team's laboratory studies vertebrate development and evolution using mouse as a model system. As a part of the TAS team, the members would set up a state-of-the-art mouse genetics facility for the generation of transgenic and knockout mice. With closer phyletic relationship to humans, mouse is a key experimental model to understand normal mammalian physiology, disease and is indispensable to study stem cell biology and regeneration. The facility, which will provide a fillip to research on campus, is a central element of the National Mouse Resource Centre (NaMoR) being developed on our campus with funds from Department of Biotechnology.

Vertebrate head is a striking addition to the body plan of chordates (animals with notochord – tissue forming the body axis). This new acquisition conferred active predatory lifestyle on vertebrates as opposed to their filter-feeding chordate relatives and contributed to rapid diversification and successful colonisation of diverse ecosystem. The head of the vertebrate is considered 'new' since much of it arises from embryonic cell types not present in other chordates. Paired sensory organs are generated from ectodermal placodes (thickenings), while the associated neural circuits, supportive skeleton, etc. derive from cranial / head neural crest (CNC). Both these cell types are unique to vertebrates. Cranial mesoderm (CM), which generates head muscles, is also likely to be vertebrate-specific embryonic tissue. How such novel cell types originate during evolution is an important fundamental question that remains largely unaddressed. The team hypothesises that co-option of specific developmental gene regulatory networks (GRN) underlie the evolution of these novel developmental stem cell types. Typically, GRN constitute a hierarchy of transcription factors acting in concert with signaling inputs. To test the hypothesis, the team would use mouse genetics approach to trace expression as well as perturb function of candidate genes of the GRN. Our studies will address the mechanisms through which these novel cell types arise and shed light on the evolution of the vertebrate head.

In the developing embryo, diverse cell types arise by the progressive lineage restriction of stem cells. The vertebrate trunk mesoderm is a versatile developmental stem cell, which generates a large variety of adult cell types. For many of the adult cell types, the downstream differentiation pathways are well-studied. However, a global perspective of gene circuits that regulate the generation of committed stem cell/progenitors from the multipotent stem cell population remain largely obscure. The team aims to map the overall design of GRNs that control the fate of early mesodermal multipotent stem cells. The team employs a combination of genome scale approaches, such as ChIP-sequencing to identify the targets of upstream mesoderm transcription factors as well as mouse genetics to map the upstream mesodermal GRN. Directed differentiation approaches to commit pluripotent stem cells to a specific cell fate are key to make stem cell therapy a reality. Knowledge of the gene regulatory networks (GRNs) that govern lineage commitment during embryonic development is crucial to develop such approaches. This work will provide clinically relevant insights into the fundamental developmental mechanisms governing organogenesis.



HONOURS AND AWARDS

Ramalingswami re-entry fellowship to establish a research laboratory in an Indian institute from Department of Biotechnology, Government of India. From 2012 to 2016

MEETINGS ATTENDED

- Invited speaker in 2nd Indian Ocean Rim Muscle Colloquium, NCBS, Bangalore, February 6-August 8, 2012.
- Invited Speaker at the Workshop on Developmental meschanisms and Model organisms, organised by the Indian Society of Developmental Biologists, Jaipur, February 24-25, 2012.
- Ramalingswami Fellows' Conclave, Hyderabad, March 11-14, 2012.
- Ramalingswami Fellows' Conclave, Thiruvananthapuram, January 20-23, 2013.
- Presented poster at Young Investigator Meet, Jodhpur, February 10-13, 2013.
- Organizer and instructor at inStem Workshop on Mouse Embryology March 11–23, 2013 (see attached flyer).
- Gordon research conference on Neural Crest and Placodes. Stonehill College, Easton, MA, USA July 21–26, 2013.
- Ramalingswami Fellows' Conclave, Pune, September 12-15, 2013.

INVITED LECTURES

- Regulatory cell state of the developmental founder stem cells of adult muscle satellite cells. 2nd Indian Ocean Rim Muscle Colloquium, inStem / NCBS, Bangalore. February 2012
- Gene regulatory circuits governing stem cell fate and development. Annual meeting of Indian Society of Developmental Biologists, Jaipur. February 2012
- Gene regulatory networks governing stem cell fate and development. Indo-Brazil Workshop on Biomedical Sciences, Varca, Goa. September 2012

LAB MEMBERS:

- Vishal Menon, Research Assistant
- Jayashree Vijay Thatte, Research Assistant
- Dibyendu Dutta, Postdoctoral Fellow
- Utkarsh Kapoor, Research Assistant
- Manjunath J., Mouse Genetics Facility Assistant

COLLABORATIONS

Ruchi Bajpai, Asst. Professor, Programmes in Biomedical and Biological Sciences, University of Southern California, Los Angeles

Virginia Papaioanou, Professor, Department of Genetics and Development, Columbia University Medical Centre, New York

Dasaradhi Palakodeti

Research Area: Stem cells and regeneration in Planaria.

Research Description: A wider understanding of stem cell function and regeneration is hampered by the lack of a tractable and simple model system. The team's laboratory uses Planaria and *Hydra*, to examine fundamental questions in stem cell and regenerative biology. Their ability to regrow the whole body or lost tissues and organs, makes these animals an excellent model system to study regeneration and stem cell function. However, the lack of well-characterised stem cells, *in vitro* culture system and transgenics have hindered the use of these model systems for studying the regeneration phenomenon.

Planaria is a bilaterally symmetrical fresh water living platyhelminth. They have specialised pluripotent cells called neoblasts that are the only dividing cells and are essential for regeneration in planarian. The primary focus of her lab is to study post-transcriptional regulation in these cells. The team's studies involve the identification, biogenesis and characterisation of small RNAs, such as microRNAs, piRNAs, and their role in neoblast function. The team is also looking at the role of different classes of RNA binding protein in stem cell function and regeneration in planaria. Hydra is a diploblastic fresh water living Cnidarian. They have unique capacity to regenerate from the dissociated cells. The team's lab is interested in studying the role of small RNAs such as miRNAs, piRNAs and endosiRNAs in cell migration, body axis formation and tissue organisation, which are the essential processes that drive *Hydra* regeneration from the dissociated cells. Further in collaboration with other members of TAS team, she also proposes to develop tools such as transgenics, *in vitro* culture of stem cells that enable these models to be used effectively to study stem cell function and regeneration in a greater detail.

LAB MEMBERS:

- Dhiru Bansal – Senior Research Fellow
- Vidyanand Sasidharan – Senior Research Fellow
- Pranavi Dasari –Junior Research Fellow
- Deepak Poduval Balakrishnan – Junior Research Fellow
- Aparna Nair – Junior Research Fellow
- Vairavan Lakshmanan – Junior Research Fellow

RECENT PUBLICATIONS:

- Krishna S, Nair A, Cheepudi S, Poduval D, Dhawan J, Palakodeti D,* Ghanekar Y.* "Deep sequencing reveals unique small RNA repertoire that is regulated during head regeneration in Hydra magnipapillata." Nucleic Acids Research2013, 41 (1), 599-616. (*co-corresponding authors)
- Sasidharan V, Lu YC, Bansal D, Dasari P, Poduval D, Seshasayee A, Resch AM, Gaveley BR,* Palakodeti D.* "Identification of neoblast and regeneration specitic miRNAs in planarian Schmidtea mediterranea." RNA2013 (in press) (*co-corresponding authors)
- Rangaiah K,* Palakodeti D. "Comprehensive analysis of neurotransmitters in regenerating planarian extract using UHPLC-MS/SRM." Rapid Communications in Mass Spectrom2013 (in press). (*corresponding author)
- Resch AM,# Palakodeti D, # Lu YC, Horowitz M, Graveley BR. "Transcriptome analysis reveals strain-specific and conserved stemness genes in Schmidtea mediterranea." PLoS One2012, 7 (4), e34447. (#equal contribution)
- Resch AM, Palakodeti D.* "Small RNA pathways in Schmidtea mediterranea." International Journal of Developmental Biology 2012, 56 (1 3), 67-74. (*corresponding author)

Akash Gulyani

Research Area: Probing cellular dynamics

Research Description: Employing a highly collaborative, interdisciplinary, and team-driven approach, the teams seek to address the mechanisms that control cell fate and behaviour using fluorescent imaging, optical techniques, and tailored molecular probes. Cellular output is very precisely regulated by highly dynamic and interconnected signaling networks. But, how is this precision achieved? Among other ways, the intra-cellular activation of signaling proteins, which make up these networks, is tightly regulated in space and time. An important focus of our research is to build tools which would help visualise, through fluorescence imaging, the precise spatio-temporal dynamics of protein activation in living cells. The teams then employ these reporters in a systems biology approach (with close collaborators) to help build a quantitative and composite understanding of cell signaling. The team has recently pioneered a new approach of using highly versatile and modular scaffolds to generate fluorescent biosensors that are engineered for live cell imaging of signaling dynamics. The idea is to screen libraries of engineered scaffolds to find binders against specific targets, and then use these binders in a modular fashion to build fluorescent biosensors that can report protein activation. This approach is generally applicable, and multiple sensors can be built using the same design strategy. The team is also setting up a programme in using chemical biology and high-throughput imaging to build novel biosensor platforms using small molecule libraries as well. This will significantly broaden the repertoire of probes for different targets. At the same time, the team is using multiple imaging modalities to enhance biosensor specificity and sensitivity. More broadly though, the team is always seeking new ways to build tools for cell biology, with the motto – if there is a fluorescent signal, there is information! The

team's quest for new tools is essentially driven by its interest in addressing basic questions in cell biology, with a special focus on cell migration and cell adhesion (binding of cells to extra-cellular proteins). These processes play critical roles in development, homeostasis, and regeneration along with diseases such as cancer, inflammation, and heart ailments. The team is specifically looking at how key signaling proteins such as the Src family kinases regulate adhesion initiation, turnover, and plasticity. In collaboration with other colleagues at inStem, the team is also applying fluorescent reporters to build a quantitative understanding of migration and adhesion signaling in epithelial and stem cell biology. The team has initiatied exciting new research in close collaboration with other members of the TAS team in using optical probes and imaging to understand cell migration and tissue remodeling in emerging models of regeneration, *Hydra* and *Planaria*. Cell migration and adhesion dynamics are a critical part of any process of regeneration and in this context - *Hydra and Planaria* (with their remarkable regenerative abilities) can serve as excellent model systems to help understand the basic mechanisms associated with tissue remodeling and stem cell fate determination. The collaborative institutional environment, interconnectedness within research teams, and the ability to leverage the collective expertise available within the TAS team and inStem/NCBS makes this effort particularly rewarding and exciting for us.

LAB MEMBERS:
Nishan B.S. – Junior Research Fellow
Sreeram Udayan – Junior Research Fellow
Ananya Mukherjee – Junior Research Fellow

Jeff Abramson

Research Area: Membrane proteins and Biophysics

Research Description: Membrane transport proteins are responsible for many critical biological functions including governing energy transduction, modifying ion concentrations, and actively importing metabolites into the cell. Membrane proteins represent ~30% of all proteins in each of the sequenced genomes. In addition, they are targets for over 50% of all marketed drugs.

Considering their biological and pharmacological relevance and their vast numbers throughout genomes, there is an enormous demand for structural information. However, membrane proteins represent less than 1% of the protein structures in the Protein Data Bank. The reason for this discrepancy stems from the hydrophobic nature of membrane proteins, which reside in a phospholipid bilayer, making them difficult to express, purify, and crystallise.

His research programme has two main foci: development of innovative tools and approaches for determining 3D structures of membrane proteins and employing these and other biochemical/biophysical tools to determine the biological basis for membrane transport. This is an ideal format for students and postdocs to interact with other groups and learn numerous techniques through interdepartmental collaborations.

LAB MEMBERS:
Vinod Nayak – Visiting Fellow
Jay Prakash Kumar – Junior Research Fellow
Patterson Clement – Junior Research Fellow

RECENT PUBLICATION

Abramson J, Vartanian AS. Watch water flow. Science 2013, 340 (6138), 1294-1295.

Kouichi Hasegawa

Research Area: Human pluripotent stem cell generation, renewal, and differentiation towards regenerative medicine

Research Description: The goal of regenerative medicine is to repair or replace damaged or diseased tissues or organs. The discovery of human pluripotent stem cells (hPSCs), embryonic stem cells (hESCs), and induced pluripotent stem cells (iPSCs) has opened up the possibility for transplantation therapy and drug screening as well as disease mechanism studies.

hPSCs retain the potency to differentiat into almost all cell types in human embryo and adult body, ability for unlimited growth with normal genetics. This hPSC character indicates their highest potential in regenerative medicine, drug development, study of disease mechanisms, and human development. Today hPSC research is a highly active area of science. The technology for derivation, propagation, and differentiation of hPSC is constantly being refined and improved, providing opportunities to not only optimise development of the cells for clinical use and drug development but also basic science of human biology and disease mechanisms. It is expected that the availability of hPSC technology will enable many programmes at inStem to dissect stem cell science and regenerative medicine.

Kouichi Hasegawa's group will establish and manage the hPSC facility in inStem for science and regenerative medicine including stem cell potency, reprogramming, differentiation, and cell engineering. The inStem hPSC facility will be a central hub for the provision of knowledge, resources, and state-of-the-art technology to researchers working with hPSC. The team primarily aims to facilitate stem cell and biomedical research at inStem and beyond to develop new technologies for working with hPSC. The team will offer hPSC line quality-controlled cell stocks and regents, technical assistance, training and education, and centralising new hPSC technologies and consultant biomedical research and development with hPSCs.

LAB MEMBERS:
Maki Murata-Hori - Visiting Scientist
Pablo Bora - Junior Research Fellow
Subhanwita Sarkar - Junior Research Fellow
Manish Kumar Singh - Junior Research Fellow


Bisleri

6 Programme on Adult Stem Cell Potency

Efforts to uncover the molecular basis of potency or 'stemness' have focused primarily on the regulation of ES cell pluripotency. While it is likely that epigenetic pathways governing potency in embryonic and adult stem cells will involve similar global mechanisms, the hierarchies and players in potency networks for adult stem cells are presently poorly understood. In addition, self-renewal in adult stem cells is associated not with proliferation as in ESC, but with the ability to enter a quiescent state. Activation, tuning, and replenishment of quiescent endogenous adult stem cells by exogenously administered agents require a thorough understanding of stem cell properties and dynamics. The core tenet of this programme is that signal-dependent epigenetic reprogramming of adult stem cells is a key modulator of stem cell proliferative capacity and potency, and that control of these epigenetic states is central to future therapeutic applications using endogenous stem cells for regeneration. The programme is working to define and link chromatin and transcriptional states to specific stem cell niche signals as a means of establishing points of intervention. The programme's strategy combines genome-scale interrogation of chromatin, transcription, and signaling in adult musculo-skeletal stem cells with targeted analysis of specific nodes/molecules. The long-term goal of this programme is to manipulate these nodes to generate predictable and controlled deployment of stem cells in homeostatic and pathological conditions, and their subsequent self-renewal after repair and replenishment are completed.

THEME MEMBERS

Jyotsna Dhawan
Senior Professor
Dean, inStem

She is interested in the biology of quiescence as a key to understanding adult stem cell function, using muscle stem cells and mesenchymal stem cells as models. Contrasting regulation in adult and embryonic stem cells has led to new insights into the role of quiescence in potency.

Programmes, Goals and Research Progress

Adult stem cells exist in most tissues and contribute to their homeostatic maintenance as well as repair/regeneration to varying degrees. These functions are compromised under pathological conditions. Strategies for stem cell replacement in a variety of acquired and inherited disorders are largely focused on transplantation of enriched populations of diverse adult stem cell

Boudewijn Burgering
Professor at University of Ulrecht, Collaborative Science Chair
His lab investigates signaling mechanisms in the control of cancer. His collaborative work at inStem is focused on transcriptional control of quiescence regulators.

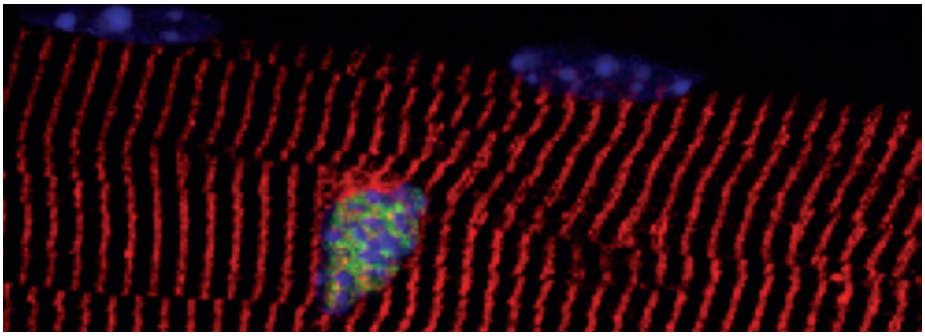
types, but in most cases are yet to show any benefit. The more challenging approach is to activate endogenous stem cells to proliferate, repair, and self-renew by using exogenously delivered molecules. For this long-term goal to be realised, the fundamental properties of adult stem cells, their molecular regulators, dynamics, sensitivities, and fluxes need to be better understood. Towards that goal, focus has been on the networks that regulate adult stem cell behaviour and properties, and propose to link intrinsic states to extrinsic signals so as to eventually control their deployment for regenerative repair.

Musculoskeletal tissue stem cells, collectively known as mesenchymal stem cells (MSC), can be isolated from skeletal muscle, cardiac tissue, and bone marrow. MSC are maintained in a quiescent state within the tissue. Unlike the cell division cycle whose complex regulation is well-defined, quiescence is poorly understood and has been largely considered to result from the decline of genetic and metabolic pathways. However, quiescent SC must exercise active quality control if they are to fulfil their regenerative role when activated. The programme's recent work provides evidence that quiescent state is conserved, signal-dependent, and actively regulated encompassing programmes beyond mitotic arrest. The broad goal of the Stem Cell Potency programme is to identify mechanisms controlling the transition of different types of MSC between quiescence and the proliferating state, and uncover targets for specific signaling molecules with the aim of increasing tissue regeneration.

The goals of the programme are:

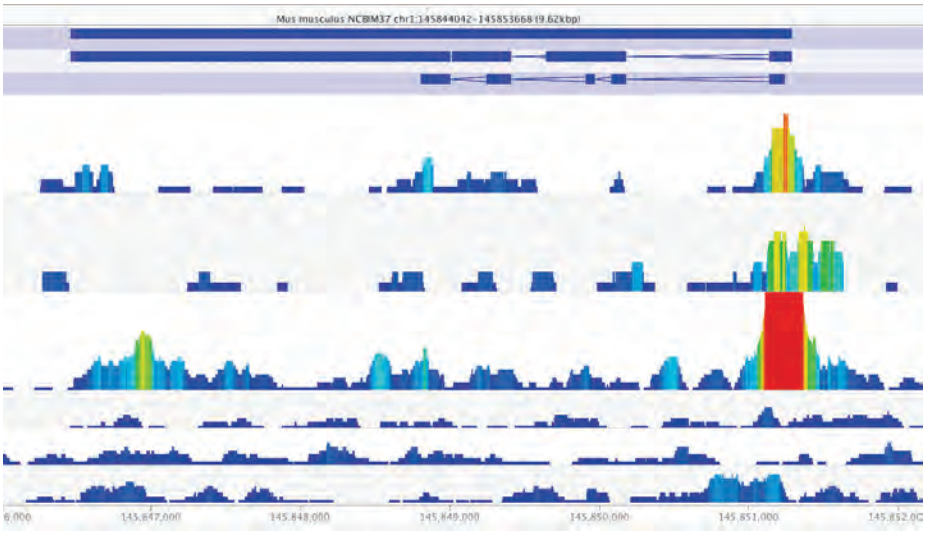
- To define the chromatin and transcriptional networks that control the maintenance, self-renewal, and deployment of adult mesenchymal stem cells in homeostasis and regeneration
- To link particular chromatin states to specific signals in the context of quiescence
- To discover modulators of chromatin states using siRNA/small molecule screens *in vitro*, based on state-specific reporter assays

The long-term goal of the programme is to modulate quiescence-dependent chromatin states in engineered mouse models *in vivo* using identified exogenous agents.



Programme 1.
Defining the chromatin state and its regulation in quiescent cells
(i) Role of H3K9Me2 transferase PRDM2 in self-renewal: This tumor suppressor was previously identified in the lab as a key chromatin regulator of quiescence in cultured muscle cells (Cheedipudi, Puri et al, submitted). Current work investigates the molecular mechanisms of PRDM2 function

in myoblasts, satellite cells, hMSCs, and ES cells using knockdowns, over-expression, and interactome analysis [A. Saleh, M. Rumman, H. Gala, L. Zhaveri, R. Arora]. Further, the team is generating PRDM2 targeted alleles in mice (using standard targeting by HR in ES cells as well as using CRISPR technology) to permit (i) tracing of expression and lineage (ii) study effects on muscle regeneration after conditional inactivation [P. Chandrasekhar, U. Kapoor].



(ii) Systems description of chromatin state in different cellular conditions: The team has established genome-wide occupancy maps of PRDM2, RNA pol II, and histone methylation patterns. All three approaches indicate a distinct poised genomic state in quiescent cells and and the programme's current work seeks to address its molecular basis and the consequences for self-renewal. The working hypothesis is that this poised state may resemble the poised or bivalent state of ES cells and provide a molecular logic for the observation that adult stem cells appear to employ the quiescent state for self-renewal mechanisms [H. Gala, B. Burgering, R. Arora, P. Chandrasekhar].

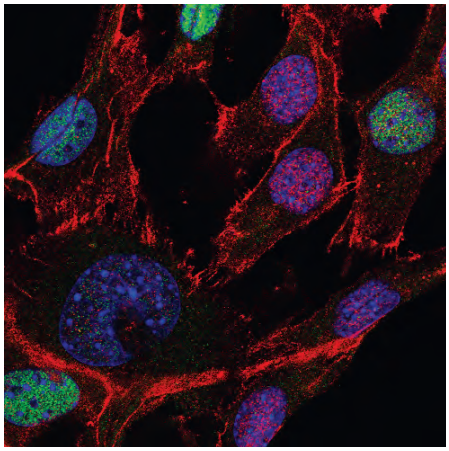
(iii) Chromatin state, redox stress, and muscle disease: Selenoprotein N (SelN) is linked to a monogenic disease, SEPN1-related myopathy, presenting with severe muscle weakness and wasting in humans. The team's collaborator, Ana Ferriero (CNRS, Paris), works with patients and has also found that SelN KO mice exhibit a loss of muscle stem cells and regenerative capacity revealing SelN as a key factor in maintaining muscle stem cells. Using a combination of *in vitro* and *ex vivo* models, the team has begun to investigate the role of SelN, associated oxidative stress, and epigenetic modifications in SC self-renewal and their response to pharmacological intervention. This new project is funded by an Indo-French grant [John Rowell].

(iv) FOXOs, quiescence, and muscle: B. Burgering, Collaborative Science Chair at inStem and Professor at Utrecht, has pioneered the understanding of FOXO transcription factors as downstream mediators of PI3K/PKB signaling in quiescence and tumor formation/suppression. Ongoing collaborative work studies the basal state of stalled RNA polymerases in quiescent cells. These findings serve as the basis for new investigations to link negative regulators of FOXO to PI3K/PKB signaling [H. Gala, A. Aloysius, B. Burgering].

Programme 2.

Linking chromatin states to signaling pathways (PDGF, Wnt, mechanical, primary cilium)

(i) PDGF and quiescence: Over the past year, the team, in collaboration with Rakesh Mishra (CCMB) and Richard Harvey (VCCRI) labs, has uncovered a PDGF-dependent poised chromatin state in quiescent cardiac MSC (Srivastava et al, in preparation). Ongoing experiments will investigate whether chromatin mechanisms found in adult skeletal muscle cells also play a role in adult cardiac MSC. This work is funded by an Indo-Australia Collaborative grant (DBT).

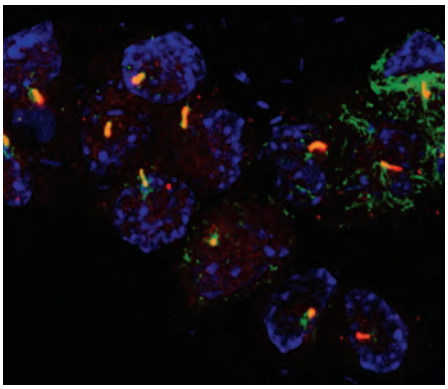


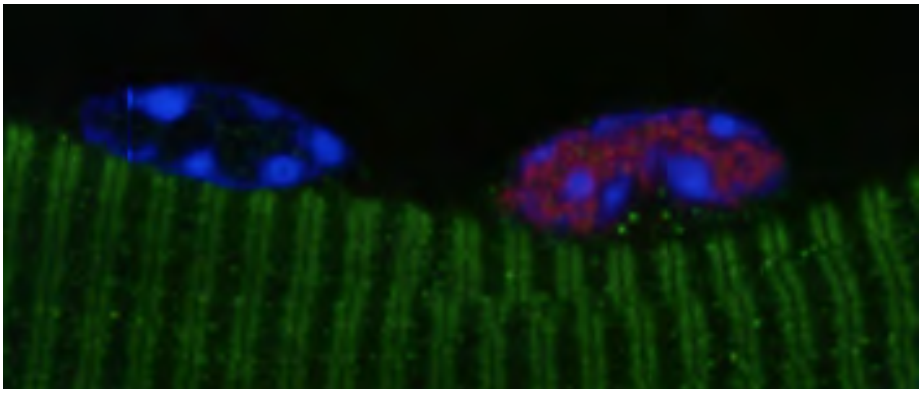
(ii) Wnt pathway and quiescence: Earlier the team identified a surprising Wnt signature in quiescent muscle cells which suggests a threshold of Wnt activation required for quiescence, distinct from that needed for differentiation (Subramaniam, Sreenivas et al, 2013). The team now has defined genome-wide targets of beta-catenin to be cell-state specific (Sreenivas et al, in preparation). In collaboration with Pia Cosma's lab (CRG, Barcelona), investigation of possible oscillatory behaviour of Wnt signaling in quiescence and effects on chromatin and transcriptional networks has begun. In collaboration with Ramanuj DasGupta of the CITH team at inStem, the team is unraveling the role of beta-catenin and TCFs in G0 [Prethish Sreenivas, Ajoy Aloysius].

(iii) Mechanical signaling: The team's earlier work established a simple model for generating quiescent hMSC using modulation of adhesion (Sellathurai et al, 2013) and surface stiffness (Majumder, Rumman et al, in preparation). In collaboration with M. Kassem and H. Schroeder labs at Odense, Denmark, genome-scale investigations are being used to define quiescence markers [M. Rumman, Malini S.P., Balu V., A. Majumder, N. Vyas]. This collaboration is funded by an Indo-Denmark grant.

(iv) Primary Cilium:

Earlier transcriptional profiling identified a signature of cilium-associated genes in quiescent muscle cells. Ongoing experiments reveal a quiescence-specific extension of the primary cilium and very rapid retraction/shedding during re-activation [N. Venogopal]. Since Wnt, Hh, PDGF, and mechanical signals are all mediated by the cilium, it is of interest to understand the mechanisms involved. An ongoing debate in the field concerns the involvement of the primary cilium in tumor suppressive mechanisms.





Programme 3.

P-bodies, miRNAs, and translational poising in quiescent cells

Quiescent cells exhibit low overall transcriptional and translational output compared to either proliferating or differentiated cells, yet rapidly activate protein synthesis when triggered to proliferate. Intrigued by the possibility that cytoplasmic P-bodies may initiate and maintain a stabilised set of mRNAs in quiescence for deployment during activation, the team is investigating the dynamics and composition of these cytoplasmic RNP granules in culture and in single muscle fibres using microscopy, biochemical purification, shRNA-mediated knockdowns, mass spectrometry, and RNA sequencing [F. Patell-Socha, N. Roy, S. Ganesh, Malini S.P., M. Rumman]. This project involves an active collaboration of H. Gowda and A. Pandey, IOB, Bangalore. Our current understanding is that P-body composition is distinct in G0 and in combination with the quiescence-specific miRNAs may contribute to translational balancing mechanisms. Ongoing investigations will reveal if inducible knockdown of P-body components alters quiescence or activation and the consequences for self-renewal.

RECENT PUBLICATIONS

- Majumder.A, Dhawan. J, Levy. O and Karp. J. M., (2012) "Applications of Microfabrication and Microfluidic Techniques in Mesenchymal Stem Cell Research" , Chapter 4, "Microfluidic Technologies for Human Health", Ed. Demirci U, Khademhosseini A, Langer, R, Blander, J., Published by World Scientific.
- Subramaniam, S., Prethish, S., Cheedipudi, S, Reddy, VR, Shashidhara, LS, Ravi Kumar Ch., MylavarapuM, and J. Dhawan (2013).Distinct transcriptional networks in quiescent myoblasts: a role for Wnt signaling in reversible vs. irreversible arrest. PLOS One.
- Sellathurai, J., Cheedipudi, S., Dhawan, J., Schrøder, H. (2013). A novel *in vitro* model for studying quiescence and activation of primary isolated/ human myoblasts. PLOS One.
- 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.
- Krishna S, Nair A, Cheedipudi S, Poduval D, Dhawan J, Palakodeti D, Ghanekar Y. (2013). Deep sequencing reveals unique small RNA repertoire that is regulated during head regeneration in Hydra magnipapillata. Nucleic Acids Res.

HONOURS AND AWARDS

- Deepika Puri (joint PhD student with Rakesh Mishra at CCMB), Nov. 2012 won the best poster award at the Asian Forum of Chromasome and Chromatin Biology: Epigenetic mechanisms of Development and Diseases
- Malini Pillai and Nainita Roy were awarded DBT postdoctoral fellowships
- Reety Arora was awarded a Start Up Research Grant for Young Scientists by DST-SERB
- John Rowell was awarded a postdoctoral fellowship under the CEFIPRA collaborative award to Jyotsna Dhawan and Ana Ferriiro
- Jyotsna Dhawan, December 2013, VASVIK Award [Smt. Chandaben Mohanbhai Patel Industrial Research Award for Women Scientists for the year 2010]

INVITED TALKS

Jyotsna Dhawan

- 'A balancing act: Chromatin regulation of quiescence' at inStem-UCL symposium on stem cells at University College, London, July 2012
- 'The Chromatin Landscape of Quiescent Cells: Implications for Stem Cell Function' at FASEB Summer Research Conference on Muscle Stem and Satellite Cells, Barga, Italy, August 2012
- 'The Chromatin Landscape of Quiescent Cells' at Mechanobiology Institute, Singapore, November 2012
- 'Quiescence and stem cell function' at Victor Chang Cardiac Research Institute, Sydney, Dec 14, 2012
- 'Quiescent Adult Stem Cells: Sleeping Beauties or High-wire artistes?' at British Society for Developmental Biology-British Society for Cell Biology
- Joint Meeting at University of Warwick, UK, March 2013

MEETINGS ATTENDED

Hardik Gala

- 'Molecular Mechanisms in Cancer' held at Cancer Genomics Centre and Centre for Biomedical Genetics, November 15-16, 2012, at Utrecht, Netherlands

Farah Patell-Socha

- Joint Meeting of the British Societies for Cell Biology and Development, March 2013 at Warwick, UK

Deepika Puri

- Presented poster at the 'Control in Biological systems' conference held at NCBS, Bangalore, January 3-5, 2013
- Presented poster at the 4th Meeting of the Asian Forum of Chromosome and Chromatin Biology, Epigenetic mechanisms of Development and Diseases November 22-24, 2012 (Best poster award)
- Presented Poster at the NIRM/ISD conference on Stem Cells, Developmentand Regulation, November 5-8, 2012, Amsterdam, Netherlands

TEAM MEMBERS

Lab Manager: Shivaji Rikka

Postdoctoral Fellows: Neha Vyas, P. Chandrashekar, Abhijit Majumdar, Farah Patell-Socha, Malini Pillai, Nainita Roy, John Rowell, Reety Arora

Graduate students:

CCMB: Prethish Sreenivas, Hardik Gala, Deepika Puri (jointly supervised by Rakesh Mishra), Nisha Venugopal

inStem/NCBS: Amena Saleh, Lamuk Zaveri, Mohd. Rumman, Ajoy Aloysius

JRFs: Dhananjay Tate, Utkarsh Kapoor, Balu Venugopal, Sravya Ganesh, Ankita Walvekar

COLLABORATORS

A. Srivastava CSCR (human MSC)

R. Harvey, VCCRI (cardiac MSC, chromatin regulation)

R. Mishra, CCMB (chromatin, bioinformatics)

P. Cosma, CRG (ES cells, reprogramming, Wnt)

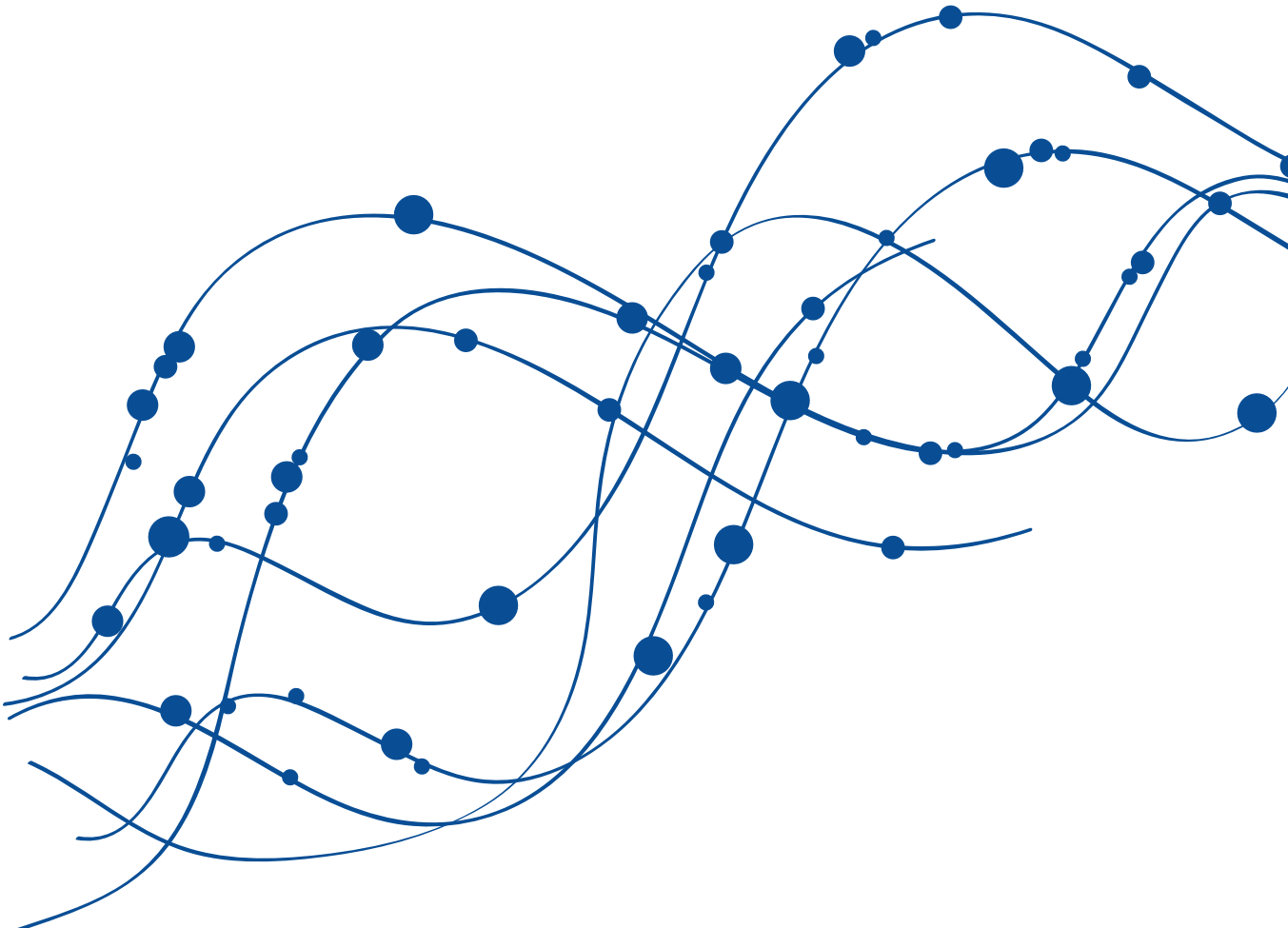
M. Kassem, H. Schroeder and J. Kjems, Odense/Aarhus (telomerized MSC, human muscle SC, antiMIRs)

H. Gowda, A Pandey IOB (P bodies, iTRAQ)

S. Pyne, Hyderabad (bioinformatics, computational biology)

A. Ansari, Wisconsin (RNA pol II regulation)

S. Hughes, P. Zammit , King's College London (P bodies in single muscle fibres)



7 Translational Research and Regenerative Medicine Programmes at CSCR

This section represents the summary of the thematic translational research programmes being developed at the Centre for Stem Cell Research (CSCR), a unit of inStem, Bangalore at the campus of the Christian Medical College, Bagayam, Vellore. 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:

(Group: At CSCR - Jayandharan G.R., R.V. Shaji, Alok Srivastava; Other collaborators: listed below under each programme)

The importance of gene therapy for treating disorders with specific genetic defects has been recognised for long. Applications have been attempted in both hereditary genetic disorders and certain acquired conditions, particularly cancers. The gene therapy programme at CSCR is being developed around application of two viral vectors. The two major sub-themes of this programme are:

a. Adeno-associated virus (AAV) vectors –

Vector Biology and Pre-clinical studies: In this area, the laboratory led by G. Jayandharan, has developed various novel AAV based delivery systems through an understanding of biology of virus and host cellular interactions. For example, an array (n=60) of 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. Indeed, translational work done in the hemophilia mouse model with hepatic gene transfer of AAV8 expressing coagulation factor IX has demonstrated their efficacy. Intellectual property protection

has also been filed for some of these vectors. Concurrently, work is ongoing on development of other innovative AAV based vector systems. MicroRNA regulated vectors or aptamer/nanoparticle coated dual AAV vector systems or generation of immune escape phenotypes and their proposed testing in hemophilia and other pre-clinical settings (eg. Leukemia) in collaboration with other investigators are some examples in this direction (see details in Jayandharan's laboratory report).

Gene Therapy for Hemophilia: Other Collaborators: Amit Nathwani, UCL, UK; Sanjay Singh, Gennova Biopharmaceuticals - Industry partner in India. More clinical collaborators from CMC will be added when the clinical programme starts.

The basic science work done at CSCR combined with the success of clinical trials conducted by Amit Nathwani from UK reported about 2 years ago using similar vectors, have paved the way for developing a clinical trial with the AAV vector/capsid modified self complementary variants of AAV8. There is a huge unmet need for the clinical management of several thousand patients with hemophilia in India who cannot receive prophylactic clotting factor concentrates lifelong at high doses due to economic reasons. Given the fact that consistent expression of 3-5% at least of FIX expression has been seen in nearly all the patients treated so far with this vector in the UK trial and with almost no significant clinical toxicity in nearly 2 years of follow-up of the first patient, there is huge scope for adopting this technology for conducting the team's own clinical trials of gene therapy in hemophilia. Towards this end, Alok Srivastava, is working on the following aspects:

- Developing the process for review, approval, and monitoring of such research proposals
- Production of GMP grade vector in collaboration with industry
- The development of the clinical trial protocol (see details in Alok Srivastava's report)

b. Lenti viral vectors for thalassemia gene therapy –

Other collaborators: Trent Spencer, Emory University, USA, and Fulvio Mavilio, Genethon, France

A collaboration has been established with the gene therapy group at Emory University, USA for developing lenti viral vectors that could be used for gene therapy of thalassemia. The origin of this collaboration lies in the fact that this group had got in touch with Alok Srivastava nearly 2 years ago to set up a collaboration for a phase I gene therapy trial for hemophilia A using lentiviral modified autologous hematopoietic stem cells for which they have already filed an IND with the USA FDA. During these discussions, the possibility of developing of using similar vector but with a different payload was broached. This has now evolved into a very vigorous collaboration with the following plan for its preclinical development:

- Vector design – This is being done jointly between the three groups involved – Emory, Genethon, and CSCR. The construct has been being developed by

Emory and is being sent anytime now to CSCR for expansion and evaluation. Based on the initial results, the plan is file for IPR on this vector. It has been designed with that aspect in mind.

- Evaluation of efficiency of the vector – This will be done by R V Shaji at CSCR using the hematopoietic stem cells (HSC) based assays for assessing hemoglobin production that he has established over the last 3-4 years. This is a unique model to evaluate this vector, including its efficacy in HSCs from patients with thalassemia, before testing them in *in vivo* models of transgenic mice with thalassemia.

Depending on the results of these studies over the next 1-2 years, including the safety profile of these vectors in terms of their random integration which will be evaluated in collaboration with other labs, further development of this work into a clinical programme will evolve. Needless to say, thalassemia is a major public health problem in India and there are huge unmet needs as well in this area. There are nearly 3-4 groups in the world now that are in active clinical trials for thalassemia using this approach and it is will be very good for us to be able to work in this area. Success with lentiviral vector based gene therapy using autologous HSCs is also being reported in various other hereditary genetic disorders. If this is successful, there will be scope to expand this into those conditions as well.

2. Musculoskeletal Regeneration Programme –

(Group: At CSCR – Vrisha Madhuri, Sanjay Kumar, Vikram Mathews, Alok Srivastava; in CMC, Vellore - Noel M Walter, Sanjay K Chilbule, Mr. Karthikeyan, Vivek Dutt, Abhay Gahukambale, Sridhar Gibikote, Balakumar, Smitha Elizabeth Mathew, Albert Abhinay Kota, Sukria Nayak, Sathya Subramani Other collaborators – (National) Jyotsna Dhawan and Ramaswamy Subramanian, Institute for Stem Cell and Regenerative Medicine, Bangalore, Prabha D. Nair, H K Varma and Annie John, from Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum. Dhirendra S. Katti and Amitabha Bhandhopadyay, Indian Institute of Technology, Kanpur, H. Krishnamurthy, and Dasaradhi Palakodeti, National Centre for Biological Sciences (NCBS), Bangalore (International). Moustapha Kassem and Henrik Daa Schroder, Lea Bjerre, Linda Harkness, and Louise Helskov Jørgensen, Odense University Hospital, Denmark

Musculoskeletal injury and dysfunction results in the more than 20% of all healthcare encounters. In India, the burden of musculoskeletal disorders accounts for 10% of the disability according to an ICMR study. New treatment and preventive strategies require collaboration between clinicians, engineers, and basic scientists. Number of cell therapies, stem cell technology, and cell/biomaterial based technologies and other regenerative products have been developed in the past decade. At present in the international scenario, there are musculoskeletal regeneration programmes in several universities such as University of Pennsylvania (UPENN) and Pittsburgh and University of California, Los Angeles (UCLA). In Vellore, a group of orthopaedic surgeons, other clinicians and scientists have now been working for several years to develop an active musculoskeletal regeneration programme.

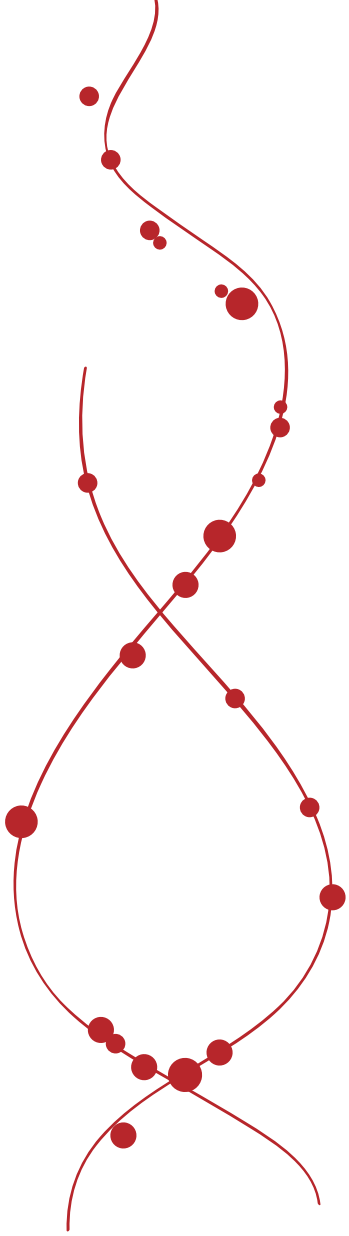
This group is actively involved in the research projects related to the regeneration of the musculoskeletal tissues such as articular and physeal cartilage, bone, and muscles. This group conducts the *in vitro* studies of the differentiated and stem cells of musculoskeletal system to translate them in small and large animals. The ultimate goal of the group is to use these cells for the regeneration of cartilage (articular and physeal), bone, and muscles in humans. The translational project relating to physeal regeneration is in its final stages while the project related to bone regeneration for long bone defects is about to commence clinical translation.

Congenital pseudarthrosis of tibia occurs in NF1 positive individuals where the bone quality is altered, there is poor bone production and abnormal activity of osteoclasts lead to bone destruction, fracture, and non-union in the tibia in affected individuals. The CPT model would allow *in vivo* exploration of therapeutic interventions. Focal and diffuse articular cartilage loss in the hip joint occurs in a number of childhood disorders. The sheep model of articular cartilage loss and replacement using scaffolds and autologous chondrocytes is being carried out to look at possible therapeutic interventions.

Simultaneously, this group also works on testing of scaffolds for the above applications and molecular diagnosis of the rare musculoskeletal disorders such as osteogenesis imperfecta, fibrodysplasia ossificans progressiva, and progressive psuedorheumatoid dysplasia.

3. Stem cell niche and cell fate programme –
(Group: At CSCR – AparnaVenkatraman, Sanjay Kumar, Murugan Ramalingam; R V Shaji, Alok Srivastava; In CMC, Vellore – Biju George and Eunice Sindhuvi (Haematology), Sukesh C Nair (Blood and marrow morphology) Marie Therese (Histopathology), Vivi M Srivastava (Cytogenetics).

There is increasing interest in the understanding of the niche of different adult stem cells that control their fate under physiological conditions from



maintaining them in quiescence to moving them towards division and differentiation. The possible role of the niche in the pathogenesis of diseases of those organs is also a subject of intense evaluation. It is the aim of this group to develop techniques and biological assays to assess the various elements of different stem cell niche components and apply them to the understanding of the pathogenesis of diseases of that organ. This programme will currently have two parts:

A. Over the last 10 years or so, the bone marrow niche of the hematopoietic stem cells has been extensively analysed. These studies have helped dissect the different cellular and molecular elements that contribute to cell fate during hematopoiesis and helped to understand the interactions between them. These studies have shown that there is an endosteal component to the niche where HSCs are generally quiescent while they are much more actively dividing at the endothelial end of the niche. Adrenergic nerve fibres also play a role in this niche as do temperature and oxygen gradients. Extension of these evaluations in transgenic animals has also shown that diseases or phenomenon which may have been presumed to be due to defects or characteristics of the hematopoietic stem cells may actually be related characteristics or defects of the niche elements.

In the first part of this programme, the team's aim to establish assays for evaluating the different cellular elements of the bone marrow niche in the normal marrow and then use those assays to assess certain diseases. In the beginning the team chose to evaluate these elements in patients with bone marrow failure syndromes for several reasons. This condition is relatively more common in developing Asian countries than in Western countries with not only the median age being almost 2 decades lower but also nearly a quarter to third of the patients being children or very young adults. This is a very different profile from what is reported in Western literature. While both genetic and environmental elements have been implicated very little is known about the cellular changes that may be contributing to these diseases. The team sees a large number of these patients of all ages and the etiology of their disease remains an enigma.

The evaluation of these patients is planned in the following manner:

- The clinical documentation of these cases will be done by Biju George and Alok Srivastava according to a standardised protocol
- Their morphologic diagnosis will be established by Sukesh C. Nair and histopathological diagnosis by Marie Therese who will also use various immunohistochemical techniques on the bone marrow trephine biopsy to mark the cellular elements in situ
- The team will also evaluate these patients for known mutations that are associated with these conditions using genomic DNA from the marrow cells as well cellular subpopulations, if needed. This work will be done under the supervision of Eunice Sindhuvi
- The hematopoietic stem and progenitor cells phenotypic, functional, and location will be evaluated by Aparna Venkatraman using FACS, *in vivo* stem and bone marrow transplantation assays and immuno-fluorescence based on her extensive post-doc work that she has done in this area.

- The mesenchymal stromal cells (MSC) will be evaluated by Sanjay Kumar using different culture, co-culture, and molecular technologies using his considerable experience in this field. He has substantial experience in this field with having worked with MSCs for over a decade. He will apply these skills to address the biology of subset of progenitor cells involved with cellular defects in bone marrow microenvironment and bone formation.

In addition to the above, 2 other scientists are also working on related areas:

- R. V. Shaji is evaluating the epigenetic basis for maintenance and differentiation of HSCs with particular reference to the erythroid lineage. This work involves assessment of adult, cord blood as well iPSC derived CD34 cells as well as CD34 cells from patients with red cell and hemoglobin disorders.
- Murugan Ramalingam is able to create artificial 3D nanomaterial environments of different chemical gradients to evaluate cellular growth characteristics. This model will also be utilised in these cellular studies. This can be a difficult subject to study because there are many variables in the niche and the team's ability to dissect them are not always robust. Also, bone marrow failure syndromes are a heterogenous group of diseases and so the team will need to carefully select the cases that they choose to evaluate. Yet with the burgeoning information about the niche and given the team's advantage with access to the clinical material and trained people particularly in CSCR. The plan is to initially evaluate about 10 each of the low risk cases and very high risk cases (closer to the leukemic spectrum). With those preliminary data, a more elaborate project is to be developed.

B. The second part of this programme, is the assessment of the gastrointestinal stem cell niche. This work will be initiated by Aparna Venkatraman, given her background in gastrointestinal epithelial work in the past and her close collaboration with this group, for human samples, if needed. Beginning with a mouse model of ulcerative coliitis (UC), this work will aim to characterise the colonic stem cell niche and understand key mechanistic indices which influence stem cell quiescence and differentiation and then dissect their role in epithelial cell dysfunction leading to development of UC.

4. Vascular Biology Programme –

(Group: At CSCR -: Rekha Samuel, Sanjay Kumar; In CMC Vellore: Jiji Elizabeth Mathews and Santhosh Benjamin, (Obstetrics and Gynecology) Nihal Thomas (Endocrinology, Diabetes and Metabolism)and MS Seshadri (retired Professor, Endocrinology, Diabetes and Metabolism), Indrani Sen (Vascular Surgery), Paul MJ and Sukria Nayak (General Surgery), Renu George (Dermatology), Ruchika Agarwal and Debashish Danda (Clinical Immunology and Rheumatology); Other collaborators: Colin Jamora, Institute for Stem Cell Biology and Regenerative Medicine, (inStem), Bangalore), H. Krishnamurthy (National Centre for Biological Sciences (NCBS), Bangalore), Niranjan Joshi and Mohanasankar Sivaprakasam (Healthcare Technology Innovation Centre, Chennai).

The broad goal of the vascular biology programme at CSCR is to understand the cellular and molecular mechanisms involved with the interaction of human endothelial progenitor and perivascular cells that lead to functional stable vasculature *in vivo*. A major focus of the lab is examining microvascular dysfunction in Type 2 diabetes utilising *in vivo* multiphoton imaging and cranial window models in Severe combined immunodeficient mouse mice. Using placental hyperglycemia as a model to extrapolate vascular defects of Type 2 diabetes, the team also examines the blood placental barrier using ultrastructural studies, *in vitro* and *in vivo* murine models. The team uses 2 approaches to obtain vascular progenitor cells; first, exploiting human induced pluripotent stem cells (hIPS)- derived vascular endothelial cells and pericytes, and second, isolation of vascular progenitor cells from adult somatic (e.g. adipose) tissue. Another area of interest in the lab involves exploring signaling pathways that influence the interaction of vascular and epithelial progenitor cells with immune cells responsible for causing vasculopathy of systemic sclerosis (diffuse scleroderma).

The strengths of the vascular biology lab include the multidisciplinary team and the clinical relevance of the team's experimental models.

Ongoing experimental models/projects include:

- **Microvascular defects in Gestational diabetes mellitus (GDM):** Upto 10-70% of GDM women and babies develop Type 2 diabetes in India. India ranks second to China in global prevalence of Type 2 diabetes. Since access to target organs of Type 2 diabetes e.g. kidney or retina is challenging, the team utilises a 9-month-old organ, the GDM placenta to examine early changes of the vasculature in the mother and newborn. Involvement of the members: Rekha Samuel (Pathology, vascular biology, human-induced, pluripotent stem cell technology and *in vivo* microscopy), Sanjay Kumar (Molecular biology and gene manipulation), Jiji Elizabeth Mathews, Santhosh Benjamin, Nihal Thomas and MS Seshadri (Clinical correlation, and expertise), H. Krishnamurthy (Flow sorting), Niranjan Joshi and Mohanasankar Sivaprakasam (Engineering and Image Analysis).
- **Generating Functional blood vessels:** The holy grail of vascular regenerative medicine is creating stable and functional blood vessels *in vivo* in vascular disease. The team has been able to generate durable blood vessels in mice; from human-induced pluripotent stem cell derived vascular progenitor cells. The team is also keen on isolating vascular progenitor cells from alternate sources such as peripheral blood, adipose tissue or from walls of blood vessels and replacing animal products with substitutes such as human platelet lysate in culture conditions. Involvement of the members: Rekha Samuel (Pathology, vascular biology, and *in vivo* microscopy), Indrani Sen and Sukria Nayak (Establishing murine ischemic models of disease, and clinical correlation), H. Krishnamurthy (Flow sorting), Niranjan Joshi and Mohanasankar Sivaprakasam (Engineering and Image Analysis)
- **Vasculopathy in Systemic sclerosis (SSc)/Diffuse scleroderma:** Despite 40 years of active research in SSC, the pathogenesis is still unknown and treatment options are limited. Vascular injury in a seminal event in

pathogenesis of SSc, that contributes to significant morbidity and multisystem disease involvement. The access to a Snail transgenic murine model that recapitulates human SSc disease, human SSc samples, and the potential to manipulate specific proteins in the mouse system, provides an innovative approach to examine vasculopathy in SSc. Involvement of the members: Rekha Samuel (Pathology, vascular biology, human induced pluripotent stem cell technology and *in vivo* microscopy), Colin Jamora, (Epidermal progenitor and stem cell biology, Snail transgenic mouse model, molecular analysis), H. Krishnamurthy (Flow sorting), Renu George, Ruchika Agarwal, Debashish Danda, and Paul MJ (Clinical Correlation and Expertise).

Translational potential of our research: Utilising vascular progenitor cells in a translational setting remains a significant challenge due to inherent endothelial dysfunction in vascular disease. It is therefore imperative to use meaningful animal models and preclinical studies to examine defects of microvasculature in diseases such as T2D, before one could envisage targeting of specific cytokines, using autologous vascular cell therapy, or vascularisation of engineered tissues at the clinic.

PUBLICATIONS: 2012-13

2012

- Advancing stem cell biology toward stem cell therapeutics. Scadden D, Srivastava A. Cell Stem Cell. 2012 Feb 3;10(2):149-50.
- Development of Novel Recombinant AAV Vectors and Strategies for the Potential Gene Therapy of Hemophilia. Zhong L, Jayandharan GR, Aslanidi G, Zolutukin S, Herzog RW, Srivastava A. J. Genet Syndr Gene Ther; S1:008, January 10, 2012.
- A novel deletion of ß-globin promoter causing high HbA2 in an Indian population. Mayuranathan T, Rayabaram J, Edison ES, Srivastava A, Shaji RV. Haematologica. 2012 Sep; 97(9):1445-7.
- Inflammation-induced effects on iron-related proteins in splenic macrophages and the liver in mice. Sukumaran A, Venkatraman A, Jacob M. Blood Cells Mol Dis. 2012 Jun 15;49(1):11-9.
- High-efficiency transduction of human monocyte-derived dendritic cells by capsid-modified recombinant AAV2 vectors. Aslanidi GV, Rivers AE, Ortiz L, Govindasamy L, Ling C, Jayandharan GR, Zolotukhin S, Agbandje-McKenna M, Srivastava A. Vaccine. 2012 Jun 6;30(26):3908-17.
- Role of Molecular Genetics in Hemophilia: From Diagnosis to Therapy Jayandharan GR, Srivastava A, Srivastava A. Semin Thromb Hemost 2012; 38(01): 64-78.
- Mobilisation of bone marrow mesenchymal stem cells *in vivo* augments bone healing in a mouse model of segmental bone defect. Kumar S, Ponnazhagan S. Bone. 2012 Apr;50(4):1012-8. Epub 2012 Feb 9.
- Bone healing by endogenous stem cell mobilisation. Kumar S, Ponnazhagan S. Bone 51(3):635 (2012).
- Long-term Cultured Human Term Placenta-Derived Mesenchymal Stem Cells of Maternal Origin Displays Plasticity. Sabapathy V, Ravi S, Srivastava V, Srivastava A, and Kumar S. Stem Cells International, Volume 2012 (2012), Article ID 174328, 11 pages.

- Genetics of Haemostasis. Goodeve AC, Perry DJ, Cumming T, Hill M, Jennings I, Kitchen S, Walker I, Gray E, JayandharanGR, Tuddenham E. Haemophilia. 2012 Jul;18 Suppl 4:73-80.

2013

- Effects of Vascular-Endothelial Protein Tyrosine Phosphatase Inhibition on Breast Cancer Vasculature and Metastatic Progression. Goel S, Gupta N, Walcott BP, Snuderl M, Kesler CT, Kirkpatrick ND, Heishi T, Huang Y, Martin JD, Ager E, Samuel R, Wang S, Yazbek J, Vakoc BJ, Peterson RT, Padera TP, Duda DG, Fukumura D, Jain RK. Journal of the National Cancer Institute, 2013 Jul 30. [Epub ahead of print].
- Nuclear Factor (NF)-KB and its associated pathways are major regulators of blood induced joint damage in a murine model of hemophilia. Sen D, Chapla A, Walter N, Daniel V, Srivastava A,Jayandharan GR. J Thromb Haemost. 2013 Feb;11(2):293-306.
- Adeno-associated virus (AAV) vectors in gene therapy: Immune challenges and strategies to circumvent them. Sangeetha H, Balakrishnan B, Sen D, Kumar S, Srivastava A, Jayandharan GR. Rev Med Virol; 2013, in press.
- Improved adeno-associated virus (AAV) serotype 1 and 5 vectors for gene therapy. Sen D, Balakrishnan B, Gabriel N, Agrawal P, Roshini V, Samuel R, Srivastava A, Jayandharan GR. Scientific Reports; 2013, 3: 1832.
- Targeted modifications in adeno-associated virus (AAV) serotype-8 capsid improves its hepatic gene transfer efficiency *in vivo*. Sen D,Gadkari RA, Sudha G, Gabriel N, Sathish Kumar Y, Selot R,Samuel R, Rajalingam S, Ramya V, Nair SC,Srinivasan N, Srivastava A,Jayandharan GR. Hum Gene Ther Methods. 2013 Apr;24(2):104-16.
- Bio-Engineering of Adeno-Associated Virus Serotype (AAV)-2 Capsid at Serine/Threonine/Lysine Residues Improves Its Transduction Efficiency Both *in vitro* and *in vivo*. Gabriel N, Hareendran S,Sen D, Gadkari RA, Govindarajan S, Hussain M, Duraiswamy R, Samuel R, Srinivasan N, SrivastavaA, Jayandharan GR. Hum Gene Ther Methods. 2013 Apr;24 (2):8093.
- High-efficiency transduction of primary human hematopoietic stem cells and erythroid lineage-restricted expression by optimized AAV6 serotype vectors *in vitro* and in a murine xenograft model in vivo. Song L, Li X, Jayandharan GR, Wang Y, Aslanidi GV, Ling C, Zhong L, Gao G, Yoder MC, Ling C, Tan M, Srivastava A. PLoS One. 2013;8(3):e58757.
- Activation of the Cellular Unfolded Protein Response by Recombinant Adeno-Associated Virus Vectors. Balakrishnan B, Sen D, David S, Hareendran S, Srivastava A, JayandharanGR. PLoS One 2013;8(1):e53845.
- Optimizing the transduction efficiency of human hematopoietic stem cells using capsid-modified AAV6 vectors *in vitro* and in a xenograft mouse model in vivo. Song L, Kauss MA, Kopin E, Chandra M, Ul-Hasan T, Miller E, Jayandharan GR, Rivers AE, Aslanidi GV, Ling C, Li B, Ma W, Li X, Andino LA, Zhong L, Tarantal AF, Yoder MC, Wong KK, Jr, Tan M, Chatterjee S, Srivastava A. Cytotherapy, Volume 15, Issue 8, August 2013, Pages 986–998.
- Overcoming challenges to initiating cell therapy clinical trials in rapidly developing countries: India as a model. Viswanathan S, Rao M, Keating A, Srivastava A. Stem Cells Transl Med. 2013 Aug;2(8):607-13.

PATENTS FILED

National:

- Methods for production of recombinant peptides and protein from non-filamentous fungi and recombinant host cell thereof. 3777/CHE/2012. Vignesh N. Jayaraman, Sudhir Reddy A, Satish Kumar P. K., Sukesh C. Nair, A.Srivastava, M. A. Vijaylakshmi,
- Mutation studies of recombinant FVIII to improve stability and activity. 3778 CHE/2012. Bilgimol Joseph, Satishkumar P. K., Sukesh C Nair, A. Srivastava, M. A. Vijaylakshmi.
- Provisional patent application No. 5171/CHE/2012. Method of preparation of human induced induced pluripotent stem cells. Sanjay Kumar.
- Novel AAV vectors-Nucleotide sequence, recombinant vector, methods and kit there of.

IN 1714/CHE/2012.PRINCIPAL INVENTOR: Jayandharan GR CO-INVENTORS: Dwaipayan Sen, Sangeetha Hareendran, Nishanth Gabriel, Ruchita Selot, Akshaya K., Balaji B., Alok Srivastava [CMC, Vellore], Sudha Govindarajan, Rupali G., N. Srinivasan [IISc, Bangalore]

International:

- Novel AAV vectors-Nucleotide sequence, recombinant vector, methods and kit there of.
- PCT Application: US 13/886,241, EP13166332.0.

PRINCIPAL INVENTOR

Jayandharan GR & Co-inventors: Dwaipayan Sen, Sangeetha Hareendran, Nishanth Gabriel, Ruchita Selot, Akshaya K, Balaji B, Alok Srivastava [CMC, Vellore], Sudha Govindarajan, Rupali G, N Srinivasan [IISc, Bangalore]

Wellcome-Trust/DBT India Alliance Early Career Fellows

Neha Vyas is investigating the mechanisms by which the secreted morphogen Hedgehog is released in vesicular form and the consequences of this packaging for the range of signaling. Neha has made substantial progress in identifying distinct vesicular pools of Hh with different signaling capacity and has defined the proteomic and miRNA composition of these secreted compartments. Neha is also collaborating with other theme members in defining secreted vesicles in muscle stem cells. (With contribution from Dhananjay Tate and Ankita Walvekar)

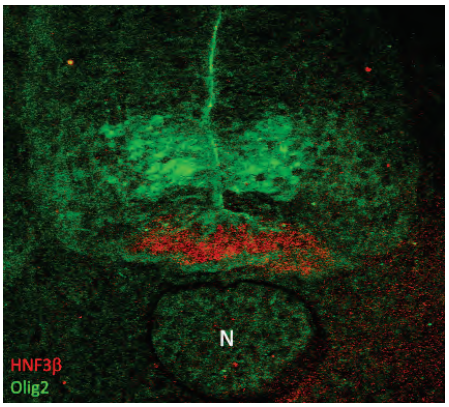
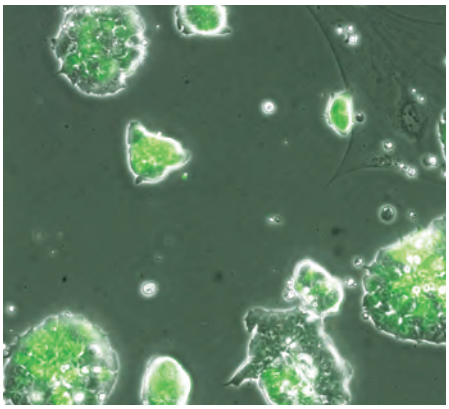


Figure 1. A whole mount preparation of chick embryo showing activation of Hedgehog target genes HNF3beta and Olig2. This assay is used to functionally define secreted vesicles containing hedgehog protein (Neha Vyas).

COLLABORATORS: Graca Raposo, Institute Curie, Paris

MEETINGS ATTENDED: 2nd HEALING International Meeting on Hedgehog-Gli, August 2013

P. Chandrasekhar is investigating the role of novel regulators of ES cell pluripotency that he identified using a bioinformatic screen. He has generated substantial background information on 4 regulators and made gene-targeted alleles in ESC, for which he is currently generating mice in collaboration with I. Chambers at Edinburgh. Chandrasekhar is investigating the signaling pathways that regulate homologous recombination (a key feature of pluripotent cells) and has developed targeting vectors for genes involved in chromatin regulation as part of a collaborative theme activity. (With contributions from Utkarsh Kapoor)



COLLABORATORS: Ian Chambers (Edinburgh), Keisuki Kaji, Institute for Stem Cell Research, (Edinburgh).

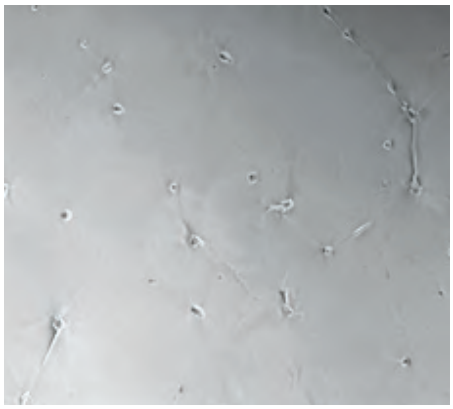
HONOURS & AWARDS

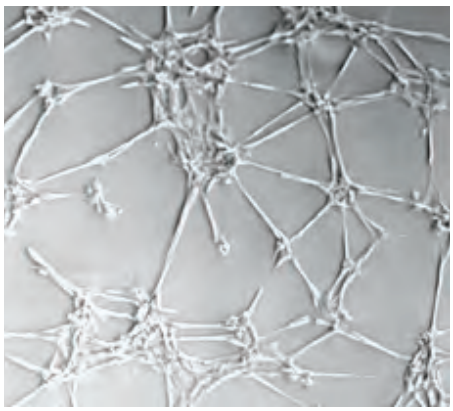
P. Chandrashekar was offered an independent faculty position at CCMB Hyderabad which he will assume in 2014.

MEETINGS ORGANIZED

Co-organised inStem Workshop on Mouse Embryology March 10-26, 2013.

Abhijit Majumder is focused on understanding the effect of mechanical micro-environment on determining cell fate, using mesenchymal stem cells (MSCs) as they offer the advantages of multi-lineage differentiation potential and therapeutic value. Abhijit has been investigating the observation that mechano-signalling is strongly coupled with cell-cell distance. While usually studied as sparse cultures, stem cells in a tissue are neither isolated nor a monolayer but are connected through the extracellular matrix. Abhijit is interested in the





mechano-response of cells when not in direct contact, but close enough to be mechanically connected via a deformable matrix. He finds that

cells can collectively modify their mechanical micro-environment and thus, can change their fate in a feed-back mechanism. (With contribution from Balu Venugopal).

COLLABORATORS: Pramod Pullerkat (RRI, Bangalore), V. Kumar (IISc Bangalore), Jeff Karp (MIT, USA)

RECENT PUBLICATIONS

- Majumder. A, Dhawan. J, Levy. O and Karp. J. M., "Applications of Microfabrication and Microfluidic Techniques in Mesenchymal Stem Cell Research", Chapter 4, "Microfluidic Technologies for Human Health", Ed. Demirci U, Khademhosseini A, Langer, R, Blander, J., Published by World Scientific, 2012
- Majumder. A., Venugopal. B and Dhawan. J., "United We Divide: Cell-Cell Force Interaction via Soft Substrate Modifies the Mechanical Microenvironment and Cell Fate", BSCB Autumn Meeting, Cumbria UK 2013
- Rumman. M, Rao. VK, Majumder. A, Venugopal. V, Pillai. M, Karp. J, and Dhawan. J, "A Soft Bed induces the Resting State: Methods to Mimic the Bio Mechanical Native State of Quiescent MSCs", 6th Mechano Biology Conference, Singapore 2012

HONOURS & AWARDS

Abhijit Majumdar was offered an independent faculty positon at IIT Bombay, which he will assume in 2014.

INVITED TALKS

- 'United We Divide: Cell Cell Force Interaction via Soft Substrate Modifies the Mechanical Microenvironment and Cell Fate' at Conference on Mechano chemical Cell Biology -British Societies for Cell Biology Autumn Meeting, Cumbria UK 2013

MEETINGS ATTENDED

- 6th Mechano-biology conference at Mechanobiology Institute Singapore, November 2012. Rumman M., Rao V.K., Majumder A., Venugopal V., Pillai M., Karp J., and Dhawan J., 'A Soft Bed induces the Resting State: Methods to Mimic the Bio-Mechanical Native State of Quiescent MSCs'
- Conference and workshop on 'Mechanical Manipulations and Responses at the Scale of Cells and Beyond" organised by NCBS MBI-ICTS, Bangalore, April 2013.

Sathya Srinivasachari is interested in deciphering the role of Rieske Oxygenases (RO) in the development of C. elegans and D. melanogaster. The primary focus of her project is on 3 ROs: DAF-36 from C. elegans (nematode worm), Neverland (Nvd) from D. melanogaster (fruit-fly), and zgc:92275 from D. rerio (Zebrafish). These ROs are known to play a major role in the early stages of steroid hormone biosynthesis, primarily in the conversion of cholesterol of dehydrocholesterol. Since the proposed RO-catalysed reaction is oxygen-dependent desaturation and monohydroxylation, neither of which is well-characterised even in prokaryotes, Sathya is keen on characterising the kinetic and thermodynamic properties of the enzyme-catalysed reaction and also in determining the structures of the proposed soluble recombinant RO proteins using X-ray crystallography.

HONOURS & AWARDS

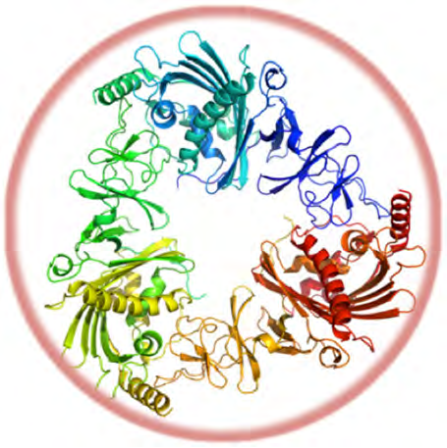
Best Oral Presentation Award in Bioengineering and Bioprospecting, Interanational Conference on Biotechnology for Innovative Applications, Amrita Institute of Biotechnology, Kollam, Kerala, August 10 – 14, 2013

INVITED TALKS

SathyaSrinivasachari, "Nanomaterials – A journey into interdisciplinary sciences", Invited lecture, National Seminar on Applications of Nanomaterials, Meenakshi College for Women, Chennai, India, 2013.

MEETINGS ATTENDED

Sathya Srinivasachari and Ramaswamy Subramanian, "Biocatalytic Role of Eukaryotic Rieske Oxygenases, DAF-36 and Nvd", Interanational Conference on Biotechnology for Innovative Applications, Amrita Institute of Biotechnology, Kollam, Kerala, August 10 – 14, 2013



Predicted 3D Model of DAF-36 and Nvd, Rieske Oxygenases from C.elegans and D.melanogaster

Shanta Wadhwani Centre for Cardiac and Neural Research

In a major boost to research at inStem, the Shanta Wadhwani Centre for Cardiac and Neural Research at inStem was established in January 2012.The institute is supported by a single philanthropic grant by the Wadhwani Foundation over a period of five years. The Centre's broad goal is to support research aimed at understanding the role of stem cells in neuroscience and cardiomyopathies. Emphasising these two goals, the Centre funds studies at the cellular and molecular level that examine neuronal and cardiac regenerative mechanisms. The international collaborative effort between inStem, NCBS, and labs at Stanford and Japan on cardiomyopathies and labs at the University of Edinburgh on neural stem cells have been made possible by the generous support provided by the Centre.

The Wadhwani Foundation is headed by Chairman Romesh Wadhwani, IT billionaire and philanthropist. Wadhwani is an alumnus of IIT Bombay and Carnegie Mellon University, USA. He currently is the Founder-Chairman of Symphony Technology Group. With its stated objective of accelerating economic development in India and other emerging economies, the Wadhwani Foundation's focus is on large-scale job creation and skill development across the country. Its endowment to inStem will achieve part of its goal of promoting innovation and excellence at centres across the country.

The Centre at inStem is focused on developing a collaborative and challenging research environment to study outstanding questions in regenerative biology. An exciting collaboration with the laboratories of Jim Spudich (at Stanford), Norio Nakatsuji, and Aki Kusumi (at iCeMS at the University of Kyoto) was the first to emerge from the Wadhwani Centre. These interactions matured in 2009-2011 to develop into a programme in cardiomyopathy led by Spudich, one in stem cell biology led by Norio Nakatsuji and the other in single molecule biology led by Aki Kusumi and Jitu Mayor of NCBS/inStem.

Building on the earlier successful establishment of the cardiomyopathy theme, 2012 has seen major advances in developing a neural stem cell research programme at inStem. Spearheaded by Sumantra Chattarji (NCBS), Siddharthan Chandran, and Peter Kind (Univ. of Edinburgh), this programme integrates neurobiologists, neural stem cell research, and behavioural physiology to address disease modeling and translational goals. The funding from the Wadhwani Foundation for such innovative collaborations allows inStem to rapidly achieve its goals in cardiac and neural research.

inStem Collaborative Science Chairs

In keeping with the collaborative philosophy of inStem, we initiated Collaborative Science Chairs (CSC) to enable rapid expansion of cutting-edge activities on campus in 2011.

Collaborative Science Chairs are accomplished leaders in their field of research and permanent faculty at institutions elsewhere in the world. CSCs work on collaborative projects with research themes at inStem, extending the scope of our activities significantly. This programme has attracted senior leading scientists to the environment for a collaborative engagement that covers 5 years of periodic visits and joint projects at inStem.

The current Collaborative Science Chairs at inStem are:

- **Jim Spudich** (Stanford), a world-renowned biophysical cell biologist has spearheaded the Programme in Cardiovascular Biology and Cardiomyopathies
- **Jeff Abramson** (UCLA), has initiated a new project in membrane protein structure as part of the TAS team
- **Boudewijn Burgering** (Utrecht), brings expertise in signaling and transcriptional control to integrate with the Programme on Adult Stem Cell Potency
- **Ashok Venkitaraman** (Cambridge), has been awarded a major collaborative grant to set up a new Centre for Chemical Biology and Therapeutics on the integrated campus
- **Norio Nakatsuji** (Kyoto), Founding Director of iCeMs Kyoto, is lending his expertise to both TAS team and Cardiac Biology team
- **Siddharthan Chandran** and **Peter Kind** (Univ. of Edinburgh), two new Collaborative Science Chairs join the Centre for Brain Development and Repair as Associate Directors, bringing expertise in neural stem cells and neuronal physiology to the multidisciplinary translational programme

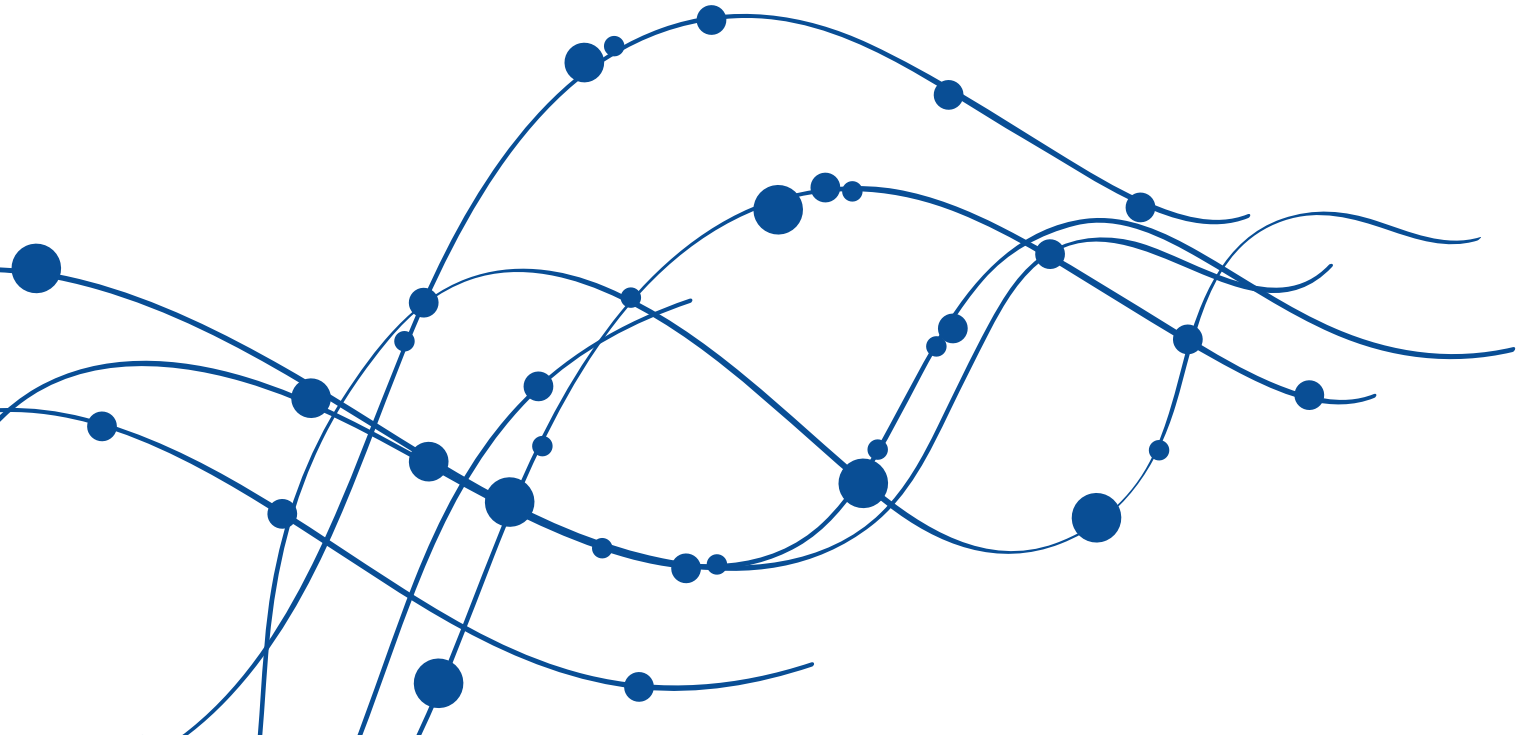
These collaborations will spearhead our interdisciplinary efforts to generate a vibrant atmosphere of international exchanges as well as to give strong impetus to our programmes on campus.

inStem Core Facilities and Campus Integration

Technology and science today go hand-in-hand. inStem strives to remain current in technology to enable its researchers in their scientific pursuits. Other than making strategic investment in instrumentation, inStem and its academic partner NCBS use C-CAMP (a section 25 company fully owned by inStem) as a vehicle to provide access to technology on campus to outside users. The state-of-the-art Next Generation Sequencing Facility is fully functional and several papers have already been published using this facility. The STED microscope development project for super resolution microscopy is well on its way.

A well-furnished Stem Cell Culture facility is in the final stages of becoming available. There will be eight clean rooms that are fully equipped to meet the needs of the investigators, four of which are already operational. The TAS team investigators in collaboration with NCBS are setting up a chemistry core facility. Other than using core funding, inStem and NCBS together have also recently succeeded in obtaining a competitive instrumentation grant to set up a state-of-the-art X-ray diffraction facility.

In summary, inStem significantly leverages the extensive core facilities already present at NCBS and enhances them. Together, the bio-cluster (NCBS/inStem/C-CAMP) reflects our unique integrative philosophy where all technologies and core facilities are available to investigators both on and off campus in a professional manner. Over 150 institutions outside of the cluster today use these facilities, and inStem and the bio-cluster will strive to continue making a larger impact.



inStem Academic Programme

Since 2011, inStem recognised the vital role of talented graduate students and postdoctoral fellows in growing the science at the facility. With a handful of faculty and a small number of young researchers, inStem initiated an informal graduate programme. This programme has now grown substantially.

The graduate programme at inStem will have at its core, strong emphasis on academic training in contemporary biology, anchored in opportunities for interdisciplinary research and training in integrated stem cell biology and allied areas. inStem faculty has been participating in team teaching of courses at NCBS since 2010 and this has grown substantially, especially in areas such as Cell biology, Developmental biology and Genomics. Students at inStem have access to course programmes on the campus as well as neighbouring institutes in Bangalore and international courses conducted on our campus. We also have active collaborative training programmes involving student exchanges, with laboratories across the world. In addition, the laboratories at inStem offer short-term learning/training opportunities to pre-doctoral students from universities and colleges within India and other countries.

As a growing institute, we aim to establish flexible ways for researchers to access training for a doctoral degree. We have joint students with faculty at NCBS and inStem from this year on; inStem is a signatory [with 11 other schools in the country] to the MoU for the joint entrance exam in biology and interdisciplinary life sciences [JGEEBILS], which will allow a new mechanism for intake of students.

This integration of a well-established and vibrant Postdoctoral Programme on campus has permitted inStem to attract outstanding young researchers with diverse background to participate in building the new integrated and collaborative research themes. The current group of ~15 post-docs includes recipients of Fellowships from the Wellcome Trust/DBT India Alliance, the NCBS-InStem Fellows Programme [with links to joint programmes with the University of Cambridge and Centre for Genomic Regulation, Barcelona] as well as competitive national schemes such as the DBT and DST postdoctoral programmes.

inStem Core Faculty

Name	Designation
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

Name	Designation
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)

inStem Leadership Committees

A. SOCIETY

- Prof. K. VijayRaghavan, Secretary to the Government of India, DBT
- Prof. M.K. Bhan, Former Secretary to the Government of India, DBT
- Prof. Satyajit Mayor, Director NCBS & Officiating Director, inStem
- Prof. Jyotsna Dhawan, Dean, inStem
- Prof. S. Ramaswamy, Dean, inStem and CEO, C-CAMP
- Prof. Upinder S. Bhalla, Dean, NCBS
- Prof. Apurva Sarin, Head Academics, NCBS
- Prof. P. Balaram, Director, IISc
- Prof. Sumantra Chattarji, NCBS & inStem
- Prof. H. Sharat Chandra, Hon. Director, Centre for Human Genetics
- Prof. K. Muniyappa, Chairman, Department of Biochemistry, IISc
- Prof. Goverdhan Mehta, Former Director, IISc & CSIR Bhatnagar Fellow
- Dr. Kiran Mazumdar Shaw, CMD, Biocon India Ltd.
- Dr. Chandrima Shaha, Director NII, New Delhi
- Dr. Chittaranjan Yajnik, KEM, Pune
- Dr. Satyajit Rath, NII, New Delhi
- Prof. Ashok Venkitaraman, Cambridge University & Adj Director, CCBT; CSC inStem
- Dr. Sunil Chandy, Director, CMC, Vellore
- Prof. Alok Srivastava, Head CSCR & Professor of Medicine, CMC Vellore
- Dr T.S. Rao, Advisor DBT
- Dr. Alka Sharma, Director & Scientist F, DBT
- Ms. Anuradha Mitra, JS & FA, DBT
- Mr. T.M. Sahadevan, Head A & F, inStem

B. GOVERNING COUNCIL

- Prof. K. VijayRaghavan, Secretary to the Government of India, DBT
- Prof. Satyajit Mayor, Director NCBS &Officiating Director, inStem
- Prof. Jyotsna Dhawan, Dean, inStem
- Prof. S. Ramaswamy, Dean, inStem & CEO, C-CAMP
- Prof. Upinder S. Bhalla, Dean, NCBS
- Prof. Sumantra Chattarji, NCBS
- Prof. Apurva Sarin, Head (Academics), NCBS
- Dr. Sunil Chandy, Director, CMC, Vellore
- Dr. Satyajith Rath, NII, New Delhi

- Prof. K. Muniyappa, Chairman, Department of Biochemistry, IISc
- Prof. Ashok Venkitaraman, Cambridge University & Adjunct Director, CCBT
- Prof. Chandrima Shaha, Director, NII
- Dr. Chittaranjan Yajnik, KEM Pune
- Dr. Alka Sharma, Director & Scientist F, DBT
- Dr. T.S. Rao, Advisor, DBT
- Prof. Alok Srivastava, Head CSCR &Professor of Medicine, CMC Vellore
- Ms. Anuradha Mitra, JS & FA, DBT
- Mr. T.M. Sahadevan, Head A & F, inStem

C. SCIENTIFIC ADVISORY COMMITTEE

2010- 2013

- Prof. K. VijayRaghavan, Director NCBS & Officiating Director inStem
- Prof. Alejandro Sanchez Alvarado, Stowers Institute, USA
- Prof. Richard Harvey, Victor Chang Cardiac Research Institute,Australia
- Prof. Thomas Rando, Stanford University, USA
- Dr. Satyajit Rath, NII, New Delhi
- Dr. Stephen Minger, King's College, London
- Prof. Shinichi Nishikawa, CDB, Riken, Japan
- Prof. Jyotsna Dhawan, Dean, inStem
- Prof. S. Ramaswamy, Dean, inStem
- Prof. Upinder Bhalla, NCBS
- Prof. Satyajit Mayor, Dean NCBS

2013-2017

- Prof. Satyajit Mayor, Director NCBS & Officiating Director, inStem
- Prof. Azim Surani, Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK
- Prof. Alejandro Sanchez Alvarado, Stowers Institute, USA
- Prof. Utpal Banerji, University of California, Los Angeles, USA
- Prof. Francesco Blasi, IFOM (FIRC Institute of Molecular Oncology, Milan), Italy
- Prof. Marco Foiani, IFOM (FIRC Institute of Molecular Oncology, Milan), Italy
- Dr. Michael A. J. Ferguson, College of Life Sciences, University of Dundee, UK
- Dr. Satyajit Rath, NII, New Delhi
- Prof. Mriganka Sur, Picower Institute for Learning and Memory, Massachusetts Institute of Technology, USA
- Dr. Mahendra Rao, NIH Centre for Regenerative Medicine, USA
- Prof. S. Ramaswamy, inStem, Bangalore
- Prof. Jyotsna Dhawan, inStem, Bangalore
- Prof. Upinder Bhalla, NCBS, Bangalore
- Prof. Apurva Sarin, NCBS, Bangalore

D. FINANCE COMMITTEE

- Prof. K. VijayRaghavan, Secretary to the Government of India, DBT
- Prof. Satyajit Mayor, Director NCBS & Officiating Director, inStem
- Prof. Jyotsna Dhawan, Dean, inStem
- Prof. S. Ramaswamy, Dean, inStem & CEO, C-CAMP
- Prof. Upinder S. Bhalla, Dean, NCBS
- Dr. T.S. Rao, Advisor, DBT
- Dr. Alka Sharma, Director & Scientist F, DBT
- Ms. Anuradha Mitra, JS & FA, DBT
- Mr. K. Kunhikrishnan, OSD, inStem
- Mr. T.M. Sahadevan, Head A & F, inStem

NON-ACADEMIC STAFF

E . ADMINISTRATIVE STAFF

- T.M. Sahadevan - Head (Administration & Finance)
- K. Kunhikrishnan - Officer on Special Duty
- K. M. Basavaraj - Project Officer
- Suresh V - Assistant Accounts Officer
- Sreenath B. A - Purchase Officer
- Archana Jain - Project Secretary
- Shobha R. - Project Secretary
- Valsala Neyyan - Project Assistant (Admin)
- Sanjay Narayana - Project Assistant (Admin)
- Aju Krishnan - Trainee (Accounts)
- Sunitha R - Project Assistant (Admin)

F. SCIENTIFIC STAFF

- Rajesh R. - Engineer C (System Administrator)
- Anand Kumar V. - Engineer C (Electrical)
- Chakrapani – Junior System Administrator
- Muneeshwaran A. – Technical Assistant (Electrical)

G. CONSULTANTS

- Wendie Ann Dockstader
- Colleen M Silan
- Shivaji Rikka
- Vijayalaxmi Nalavadi
- Aude Conscience

Design: www.pinklemonade.in

National Centre for Biological Sciences-TIFR
GKVK, Bellary Road, Bangalore - 560 065, India
Telephone: +91 80 23666001/67176001/02/18/19
Fax: +91 80 23636662,23666268
www.instem.res.in

Photo credits: John Rowell, Avinash Chinchure