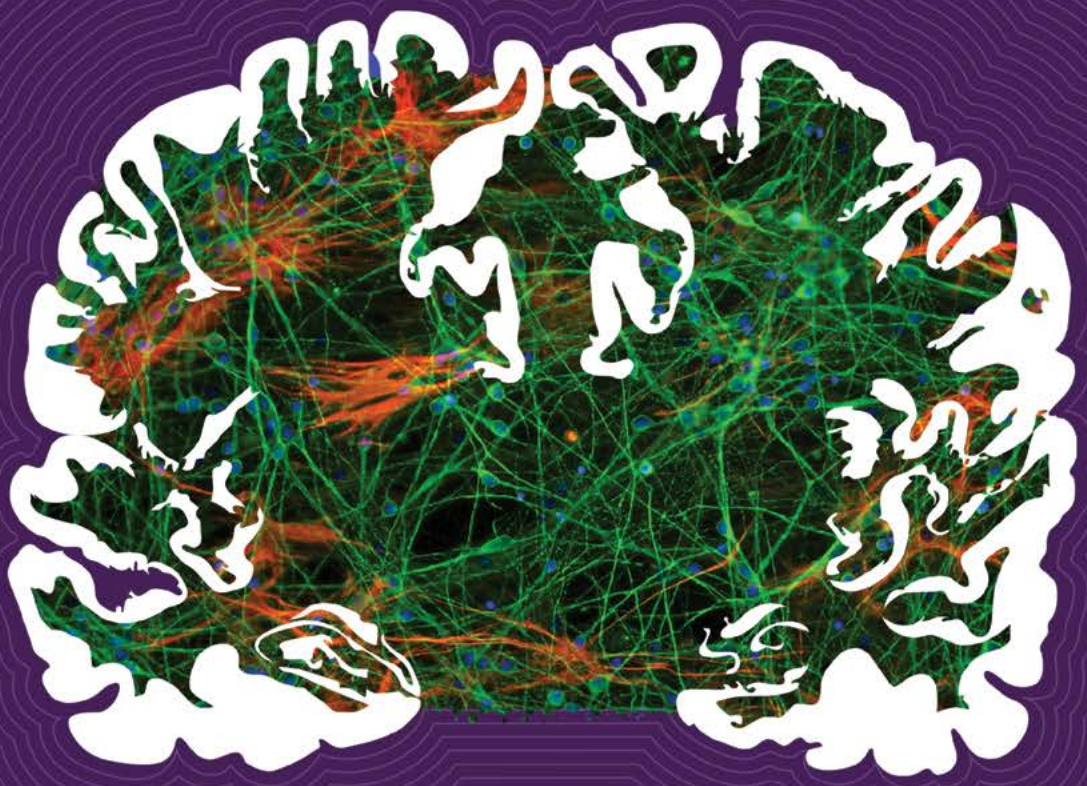
Institute for Stem Cell Science and Regenerative Medicine

ANNUAL REPORT 2015-16



inStem

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

Institute for Stem Cell Science and Regenerative Medicine

ANNUAL REPORT 2015-16

inStem

Contents

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Director's Note

There has been a whirlwind of activities at inStem in the past year, as a young institution makes its presence felt, not the least, for providing grist for this year's report.

As the culture of 'collaborative inquiry' allows inStem to grow its science distinctly, it also enables researchers to tackle major, complex scientific problems that are difficult to pursue in single investigator laboratories. This was a hopeful thought when inStem was being put together. Now, in the words of the recently concluded Scientific Advisory Board report, "The SAB noted its appreciation of the innovative core idea of inStem, namely, putting outstanding and enthusiastic young researchers into interesting small thematically focused communities. The SAB was of the opinion that in a world in which such spaces are uncommon, inStem provides a place for collaborative exploratory-translational science, including but not limited to the new-technologies-new-science interface. The SAB feels that inStem has made impressive progress over the past year and that the institutional atmosphere has been energised."

Also inserting a note of caution about how the success of individuals and such collaborative research structures may be monitored and mentored, "The SAB agreed with the Chair, inStem GB (Governing Board), that institutions such as inStem have to be in the vanguard of developing new ways and metrics of assessing career success for individual researchers in complex collaborative areas of science." This is an important issue and needs careful deliberations amongst the few programs of this nature around the world. While the tangible output that has been generated from inStem is surely one measure, I sense a growing ease in the development of a collaborative culture on the campus; inStem is also now able to attract excellent young scientists who wish to work in such an environment. With these tendrils of positive indications, I hope we will have better traction in scaling new heights at inStem, and on the whole campus at large.

Another sign of institutional growth is associated with transitions. Ramaswamy is stepping down as a Dean of inStem, and as the Administrative Head (in the absence of a full time Head, Administration). Ramaswamy rendered yeoman service to inStem to put in place an administrative structure jointly with Mr. Kunhikrishnan. Now with the end of Mr. Kunhikrishnan's tenure as a consultant, we have recruited Mr. A. N. Ramachandra as our new full time Head, Administration to shape inStem into a robust and well run institution. While welcoming Mr. Ramachandra on board, and wishing him all success, I would like to record and recognize Ramaswamy's and Kunhikrishnan's contributions to the establishment of inStem

and seeing it through its early phase. Ramaswamy's infectious energy has laid the foundations of a responsive administration that I hope will remain the hallmark of what is going to be set in place. We all wish Mr. Kunhikrishnan well in his future plans and thank him for his steady hand and mature engagement. Of course, we now can look forward to some exciting science from Ramaswamy's laboratory and the TAS team that he leads, liberated from onerous administrative responsibilities. With departures and turnovers, we are also joined by new people. Mrs Uma H.R. has taken over the management of inStem Accounts, and we look forward to her stewardship in this important area. We welcome the human geneticist, Dhandapany Perendurai; he has now joined us in body and spirit, bringing a new level of enthusiasm to the Cardiomyopathy theme. We wish him great success in all his endeavours on the campus and in strengthening links without.

Last year heralded the setting up of the Bangalore Life Science Cluster Board, and under the stewardship of Mr. Knight Paul Pandian, former Financial Advisor to the DBT and Ministry of Earth Sciences. Three institutions, NCBS, inStem and CCAMP, are working together to create an integrated campus for a whole set of activities around Life Sciences, fundamental research in individual laboratories, thematic research with a more translational focus and a potential for technology development and entrepreneurship. The formation and structure of the cluster and its governance have been a part of a major initiative of the DBT to connect institutes together to effect synergies in functioning and to enhance the critical mass of researchers and entrepreneurs who are connected up in one space. The Bangalore Cluster is unique in that it requires a partnership between two Departments of the GoI, the DBT and DAE, and the realization of this cluster is testimony to the willingness of these agencies to work together, and incentivize the same. Under this cluster initiative, a Big Data and Cryo EM facility for structural biology has been envisaged, for which we are eagerly awaiting a sanction from the funding agencies.

Without the cluster, new links are being forged, and old ones strengthened. The establishment of a major multi-institutional programme earlier this year, linking inStem, NCBS, NIMHANS, and CMC Vellore, supported by the DBT, on Accelerating the application of Stem cell technology in Human Disease (ASHD) to engage with Blood and Brain disorders, has been a milestone at inStem. Under this aegis, inStem, NCBS, and NIMHANS have come together to take on a programme of national dimensions on Accelerating Discovery in Brain Disorders using stem cells (ADBS). Collaborative Science Chair, Mahendra Rao (inStem) and Raghu Padinjat (NCBS), along with Sanjeev Jain and his clinical colleagues at NIMHANS have put together a programme that exploits the extraordinary opportunity offered by India's human genetic variation coupled with its high levels of consanguinity to explore the causes of mental illness (www.ncbs.res.in/

adbs/). Over the past years, five clinics at NIMHANS have engaged with patients with familial incidence of clinically diagnosed mental illnesses, and have recruited these families to a cohort. Deep clinical phenotyping, blood samples, stem cells and genomic information at different levels are being collected from these individuals, and unaffected family members to develop a long term study of the causes and consequences of these disorders. The encouragement and support of Kris Gopalakrishnan (Infosys co-founder) and the Pratiksha Trust set up by Kris and Sudha, have been invaluable for us to take on these challenging initiatives. With the establishment of a Centre for Brain Research at the IISc by Kris last year, and the co-location of the Centre for Brain Development and Repair (CBDR) at inStem, the ADBS provides an unprecedented opportunity for scientists and clinicians interested in understanding Brain function and Disorders, to link up with the untapped potential of human genetic variation available in our own neighbourhood. The Blood Disorders part of the AHSD programme is being spearheaded by Alok Srivatsava, and his colleagues at the Centre for Stem Cell Research (CSCR), at CMC Vellore. Here they address three main areas, one related to community health and diagnostics for inherited blood disorders, such as sickle cell anemia and thalassemia; another related to use of gene therapy in these diseases; and the third, the establishment of a haplobank. We expect to report more on these endeavors in the coming years.

Our experience with support for research from the Wadhwani Foundation towards the Shanta Wadhwani Centre for Cardiac and Neural Research (SWCCNR; see page 110) at inStem, focused on driving new discoveries in Cardiac and Brain Stem Cell Biology, and the substantial support from the Pratiksha Trust has given us a much clearer picture of how such private philanthropic support can hugely catalyze research on our campus. The role of the SWCCNR has been a major catalyst in helping to put together the ADBS programme, which in turn attracted substantial support from the DBT, and is now being enhanced by a generous donation from the Pratiksha Trust.

It is vital that such support continues to flow for us to take our science to the highest level. This year marks a new effort on our side to develop a major Endowment Fund for our campus. 2016 is NCBS's 25th year and as a major partner in the Bangalore Life Science Cluster, we are seeking Endowment Funds for institution building as well as providing flexible support for a number of initiatives to commemorate this landmark year. Bengaluru has a number of Friends of Science, and they have been very generous with their time and support. I would like to gratefully acknowledge unstinting support for this Endowment Fund from the Infosys Foundation. This is just a beginning and we hope to capitalize on our success this year to create a long term sustainable Endowment fund for the campus.

Finally, we are all eagerly awaiting our move to the new building and by this time next year we hope all the lights and action would have been switched on in this new space.

S MAYOR
Director, inStem

2

Administration Report

The Institute has completed its seventh year in its pursuit for excellence in stem cell research. The infrastructure development is in full-swing and new laboratory facilities will be available for use shortly.

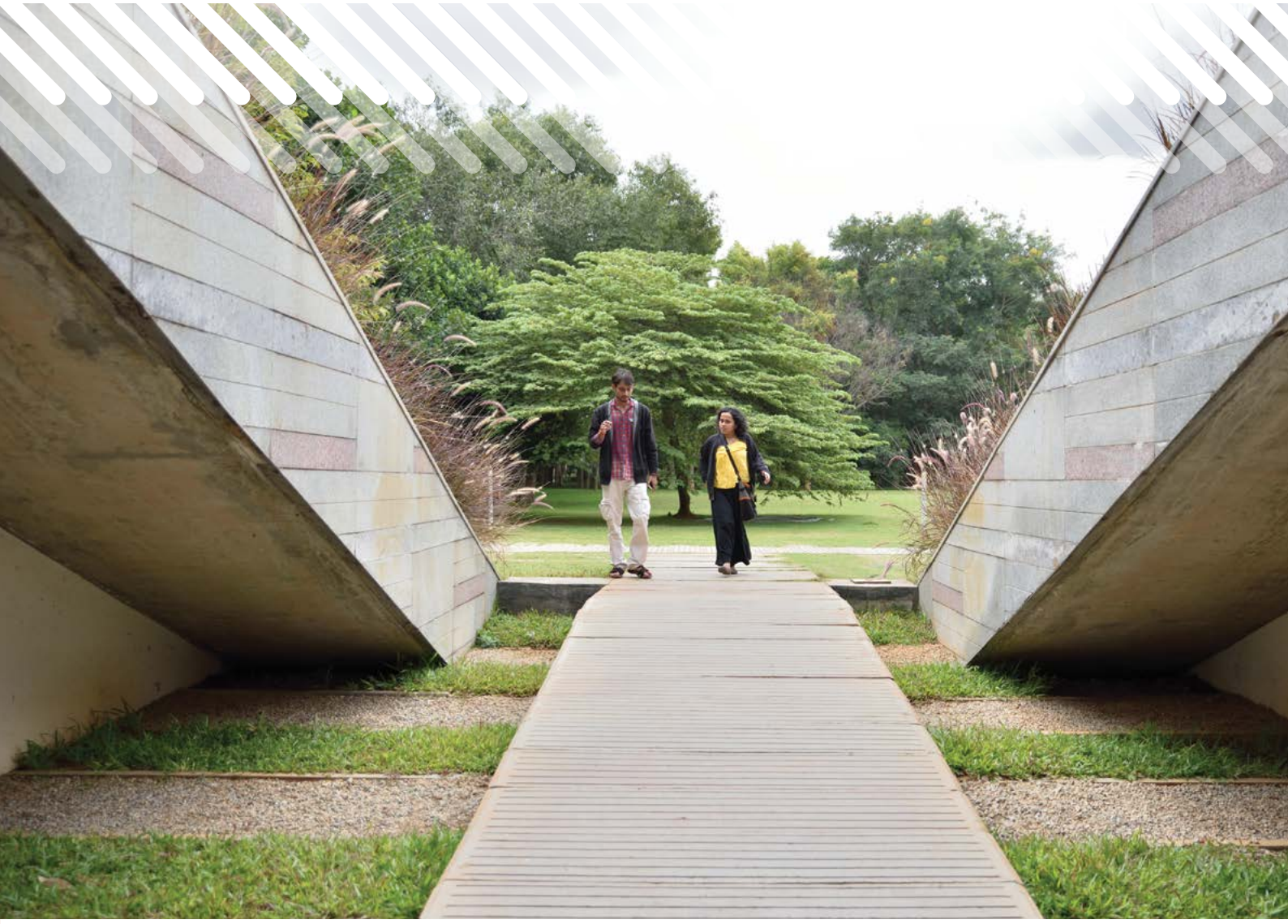
The National Centre for Biological Sciences (NCBS) and the Centre for Cellular and Molecular Platforms (C-CAMP) continue to extend shared services to inStem as participants in the Bangalore Life Science Cluster (BLiSc). This has resulted in sharing of resources and saving in costs of running independent activities in the support system. This model of shared growth is showing potential for the future.

The table below indicates the status of grants received and the manpower count:

	2014 - 15	**2015 - 16**
Core grants received	Rs. 462.2 million	Rs. 384.7 million
EMG grants received	Rs. 352.4 million	Rs. 502.2 million
No of active grants	36	50
Manpower	174	215

Systemic improvements in the functioning has accelerated the activities of the administration. Our colleagues in Scientific, Technical and Administration groups (of inStem and the whole campus) rallied to support all activities of the entire campus. The Administration team is working to make the new facilities functional as soon as possible. The Construction team is engaged in giving the final shape to the architecturally pleasing and functionally efficient Structure.
In summary, the Institute has progressed considerably during this year, paving the way for further growth in coming years.

A.N. Ramachandra
Head – Administration, inStem



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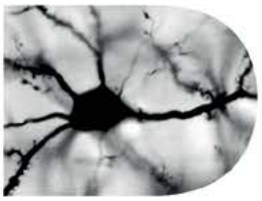

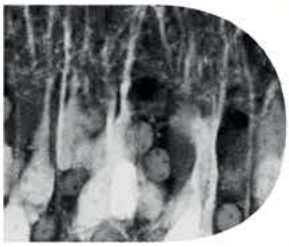

3

Centre for Brain Development and Repair

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

SUMANTRA CHATTARJI
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3.1 *Sumantra* Chattarji

CENTRE FOR NEURODEVELOPMENTAL SYNAPTOPATHIES (CNS)

Neurodevelopmental and neurodegenerative disorders represent a major and growing public health threat. Our research spans multiple levels of neural organisation and combines animal and human-based model systems to accelerate the discovery and delivery of effective therapeutics for these largely untreatable conditions.

Programme 1: Modelling human ASDs "in a dish"

SIDDHARTHAN CHANDRAN, DAVID WYLLIE, SUMANTRA CHATTARJI

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

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

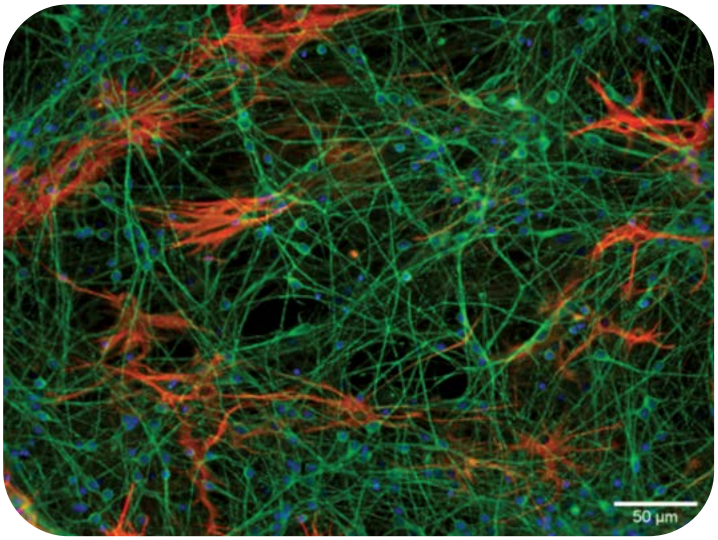

Fig 1

Human iPSC-derived wild-type cortical neurons co-cultured (8 weeks *in vitro*) on rodent cortical astrocyte feeder layer. The cells have been immune-labelled for DAPI (blue), MAP2b (green) and GFAP (orange).

Using a combination of whole-cell patch clamp recordings and microscopy, we are investigating if human cortical neurons recapitulate known physiological and anatomical milestones and (Figure 2), if so, whether these milestones are reached in human iPSC-derived neurons from affected individuals. Further, in the broader context of cellular autonomy and ASDs, we are studying the role of astrocytes in the development and function of forebrain neurons derived from FXS patient iPSC lines.

Programme 2: The autistic network – from pathways to rescue

SUMANTRA CHATTARJI AND PETER KIND

Using novel transgenic rat models of ASDs, we are addressing whether genetically heterogeneous disorders share common synaptic neuropathology, as well as whether the common synaptic pathophysiology that arises from shared "developmental" mechanisms could be a therapeutic target throughout the lifespan of the animal. For instance, do rare, highly penetrant forms of ID with co-occurring ASD share a common time-course of cellular circuit-level defects? Using multi-electrode array (MEA) recordings in brain slices we are analysing the convergence of circuit-based phenotype in the cortex of rodent models (Figure 2). We have also completed a detailed characterisation of post- and pre-synaptic defects in the amygdala of both mouse and rat models of FXS. Identification of deficits in activity-dependent synaptic plasticity in the amygdala, in turn, has enabled us to examine their functional consequences at the systems/ behavioural levels.

Programme 3: Autistic function – rat behaviour and imaging

PETER KIND AND SUMANTRA CHATTARJI

We continue to generate new rat models of highly penetrant single-gene causes of ASD/ID to better model autistic and cognitive behaviours that can accurately reflect autistic features in humans. Rats are preferable in this regard to mice as they have a wider repertoire of social behaviour. They also permit the use of functional magnetic resonance imaging (fMRI) in awake, behaving animals that then allows parallel studies in rodents and humans using the same modality. In a recently a published study from CBDR, using a new rat model of FXS, we report that *Fmr1-KO* rats exhibit elevated basal protein synthesis and an increase in mGluR-dependent

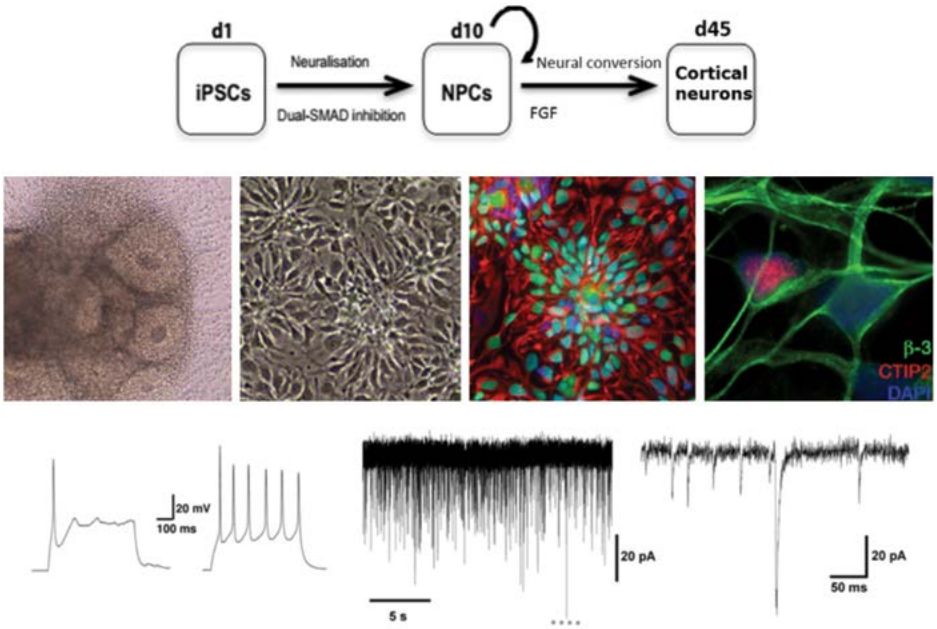

Fig 2

Excitatory cortical neurons at 28 days in vitro following differentiation from human pluripotent stem cells. Neurons have been immuno-labelled for DAPI (blue) MAP2b (green) and beta3 tubulin (grey).

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

Collaborators

Richard Morris, Mike Cousin, Giles Hardingham, Matt Nolan (University of Edinburgh); Upinder S. Bhalla (NCBS); Sanjeev Jain and Biju Vishwanath (NIMHANS, Bengaluru).

Siddharthan Chandran

Peter Kind

David Wyllie

Publications

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

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

Márkus, N.M., Hasel, P., Qiu, J., Bell, K.F.S., Heron, S., **Kind, P.C.,** Dando, O., Simpson, T.I. and Hardingham, G.E. (2016). Expression of mRNA Encoding Mcu and Other Mitochondrial Calcium Regulatory Genes Depends on Cell Type, Neuronal Subtype, and Ca2+ Signaling. ***PLoS One*** 11, e0148164.

Crocker-Buque, A., Currie, S.P., Luz, L.L., Grant, S.G., Duffy, K.R., **Kind, P.C.** and Daw, M.I. (2016). Altered thalamocortical development in the SAP102 knockout model of intellectual disability. ***Hum. Mol. Genet.***

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Barnes, S.A., Wijetunge, L.S., Jackson, A.D., Katsanevaki, D., Osterweil, E.K., Komiyama, N.H., Grant, S.G.N., Bear, M.F., Nagerl, U. V., **Kind, P.C., Wyllie, D.J.** (2015). Convergence of Hippocampal Pathophysiology in Syngap+/- and Fmr1-/y Mice. ***J. Neurosci.*** 35, 15073–15081.

Chattarji, S., Tomar, A., Suvrathan, A., Ghosh, S. and Rahman, M.M. (2015). Neighborhood matters: divergent patterns of stress-induced plasticity across the brain. ***Nat. Neurosci.*** 18, 1364–1375.

Livesey, M.R., Magnani, D., Hardingham, G.E., **Chandran, S.** and **Wyllie, D.J.A.** (2015). Functional properties of in vitro excitatory cortical neurons derived from human pluripotent stem cells. ***J. Physiol.***

Till, S.M., Asiminas, A., Jackson, A.D., Katsanevaki, D., Barnes, S.A., Osterweil, E.K., Bear, M.F., Chattarji, S., Wood, E.R., **Wyllie, D.J.A., Kind, P.C.** (2015). Conserved hippocampal cellular pathophysiology but distinct behavioural deficits in a new rat model of FXS. ***Hum. Mol. Genet.*** 24, 5977–5984.

Invited Talks

Gordon Research Conference on "The Amygdala in Health & Disease", Stonehill College, MA, USA (2015).

International Brain Research Organisation, 9th World Congress, Rio De Janeiro, Brazil (2015).

Center for Neural Science, New York University, New York, USA (2015).

Meeting of the Professional Development Committee, Society for Neuroscience, Washington DC, USA (2015).

Cold Spring Harbor, Asia meeting on "Neural Circuit basis of Behaviour and its Disorders", Suzhou, China (2015).

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3.2 *Ravi* Muddashetty

REGULATION OF PROTEIN SYNTHESIS DURING NEURONAL DEVELOPMENT AND PLASTICITY

My lab is interested in understanding the regulation of activity mediated protein synthesis in the nervous system during development and plasticity. Our current focus is on the role of fmrp during neuronal differentiation and regulation of protein synthesis at the synapse in response to mGLuR and NMDAR stimulation.

The ability to make proteins on demand is a prerequisite and a salient feature of synaptic plasticity. FMRP (Fragile X Mental Retardation Protein) together with microRNA Induced Silencing Complex (miRISC) plays a central role in this. FMRP mediates the interaction of specific components of RISC or translation machinery for a select subset of mRNAs in an activity dependent manner. This way, FMRP determines the specificity of response to neuronal activity and thus maintains the translation balance at the synapse. A major effort in the lab is to identify FMRP associated proteins and how they influence translation in response to a specific stimulus (such as NMDA, DHPG and BDNF). MOV10 is one such protein we have identified which is particularly interesting because its interaction with FMRP and AGO2 determines the subset of mRNAs to be translated in response to NMDAR stimulation (Figure 1). MOV10 was previously shown to be associated with AGO2 and involved in NMDAR mediated translation regulation. We discovered that FMRP is essential for MOV10 interaction with AGO2 and show that MOV10 interaction with AGO2 and FMRP changes in response to neuronal activity. In rat (P30) cortical synaptoneurosomes, MOV10-AGO2 interaction increases on mGluR stimulation (also in cultured cortical neurons) and decreases on NMDAR stimulation (Figure 1A). These results indicate that MOV10 forms a translation inhibitory complex with FMRP and AGO2. mGluR stimulation facilitates the formation of this inhibitory complex while NMDAR stimulation leads to its dissociation (Figure 1C).

On a linear sucrose gradient (polysome profiling), MOV10 is distributed both in lighter (along with FMRP and AGO2) and heavier fractions (polysomes). The presence of MOV10 in the polysomal fraction is sensitive to puromycin treatment indicating that while a part of MOV10 is associated

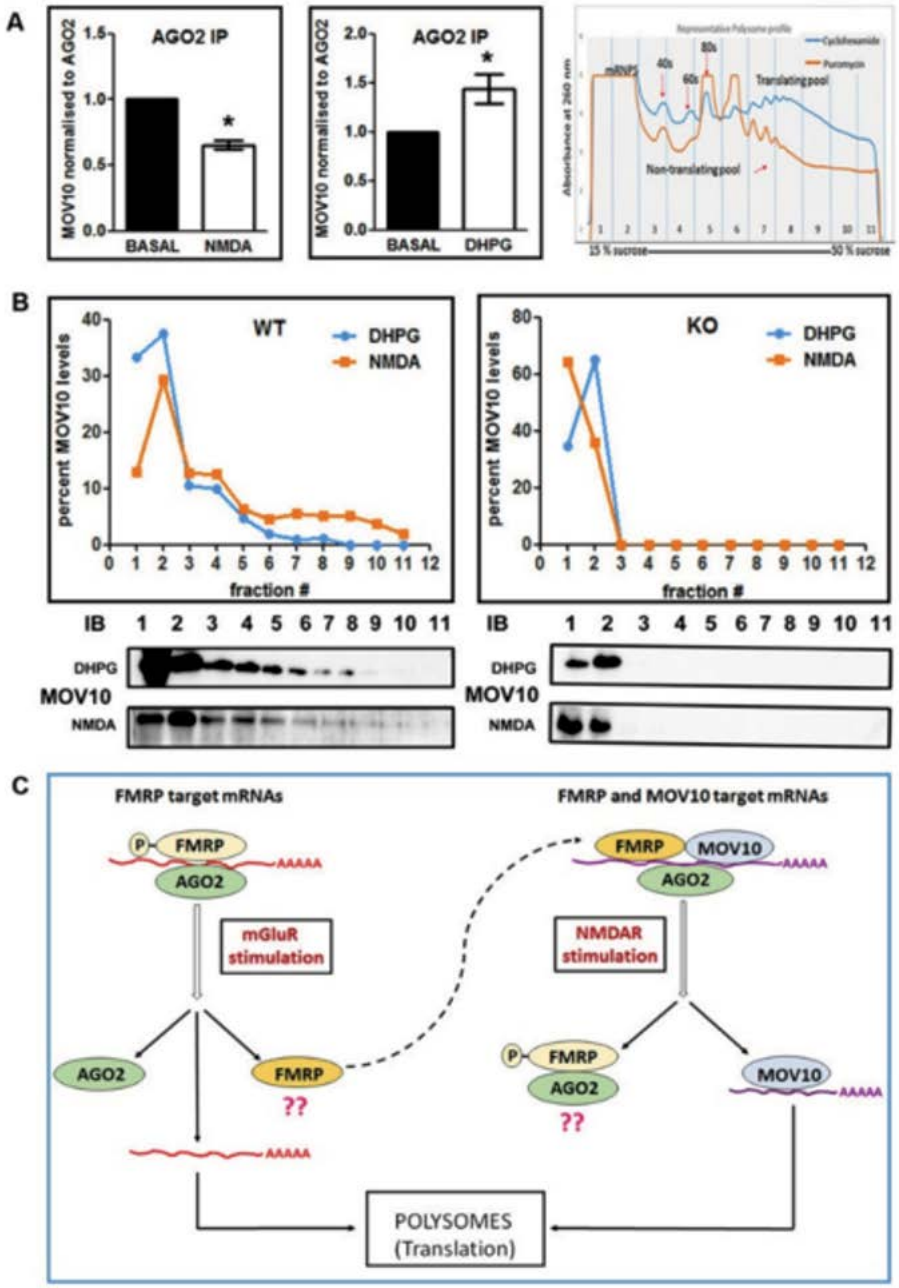

Fig 1

MOV10-FMRP-AGO2 interaction determines the translation in response to NMDAR and mGluR stimulation.

A) MOV10-AGO2 is decreased on NMDAR stimulation and increased on mGluR stimulation.

B) MOV10 moves to polysomes on NMDAR stimulation and moves out of polysomes on mGluR stimulation. This response is lost in the absence of FMRP.

C) Model to explain translation in response to NMDAR and mGluR

with translation inhibitory complex, the remaining part is associated with translating polysomes. In cortical synaptoneurosomes, on NMDAR stimulation, there was an increase in the presence of MOV10 in the polysomal fraction (in contrast to its decreased association with AGO2). Similarly, on mGluR stimulation, MOV10 presence in the polysomal fraction was decreased (Figure 1B) in contrast to its increased association with AGO2. In cortical synaptoneurosomes prepared from

Fmr1 knockout rats, MOV10 was completely absent in polysomal fractions. Since there is no MOV10 in the polysomal fraction, it also does not respond to NMDAR and mGluR stimulation (Figure 1B). In summary, MOV10 interacts with FMRP and AGO2 to form an inhibitory complex which is facilitated by mGluR stimulation. On NMDAR stimulation MOV10 dissociates from AGO2 and shifts to translating polysomes and promotes translation for its target mRNAs. FMRP is essential for both of these functions and thus in the absence of FMRP both mGluR and NMDAR mediated translation is dysregulated. Based on this, we propose a model (Figure 1C) to explain the differential translational regulation in response to mGluR and NMDAR stimulation based on FMRP phosphorylation, MOV10 and AGO2 interaction.

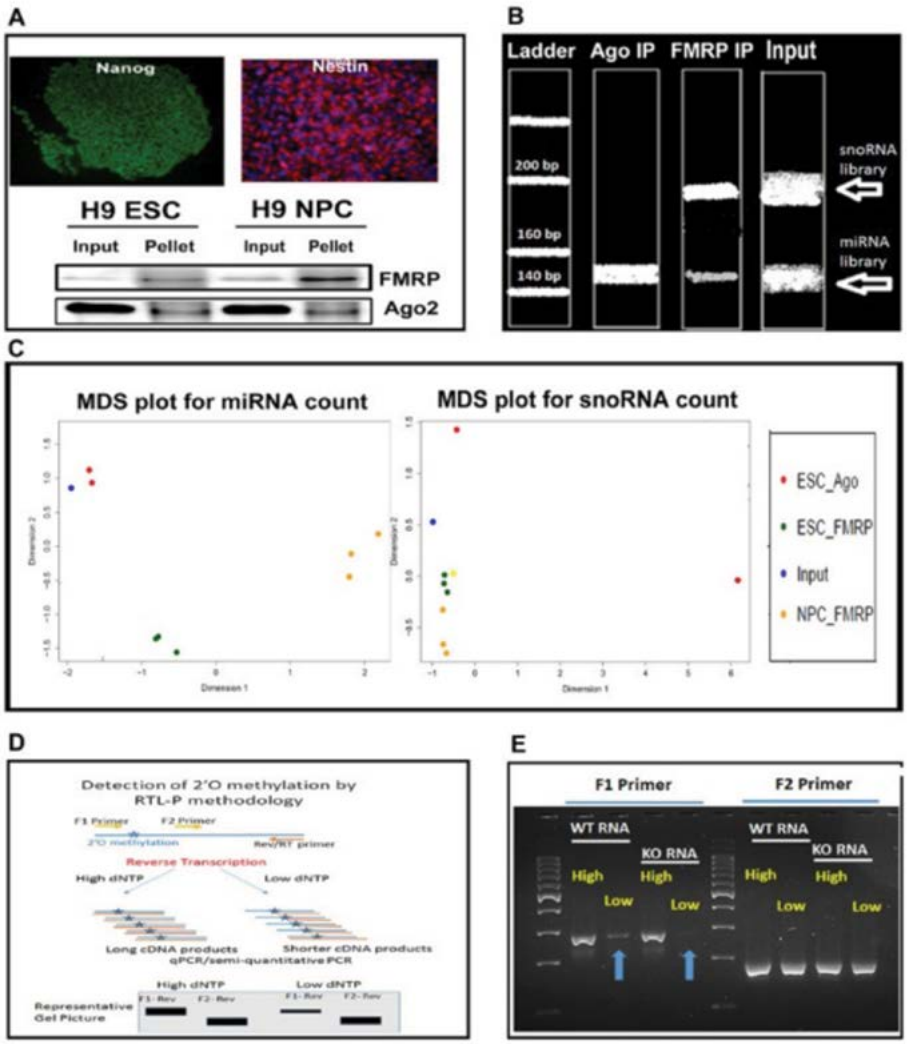

Fig 2

FMRP interacts with microRNAs and snoRNAs.

A) and B) FMRP interacts with specific sets of microRNAs and snoRNAs in both ESCs and NPCs.

C) and D) MDS plot showing FMRP interaction with distinct sets of microRNAs in ESCs and NPCs while it interacts with same set of snoRNAs in both cells.

E) Experimental design to test the extent of methylation on ribosomal RNA.

F) Increased methylation of rRNA sites targeted by FMRP associated snoRNA in FMRP-KO ES cells.

Many neurodevelopmental disorders are likely to have an origin in the neuronal differentiation programme itself. The absence of FMRP is reported to affect the process of neuronal differentiation. In this context, we decided to identify FMRP-associated small non-coding RNAs to better understand the role of FMRP in embryonic stem cells (ESCs) and their differentiation into neuronal precursor cells (NPCs). In our attempt to identify FMRP associated microRNAs, we observed a prominent higher-molecular weight band in FMRP pulldown (other than the microRNA band) which was completely absent in AGO2 pulldown as shown in the schematic (Figure 2B). To our surprise, this turned out to be a specific subset of snoRNAs (small nucleolar RNAs). Interestingly, FMRP interacts with two distinct sets of microRNAs in ESC and NPCs, while it interacts with the same subset of snoRNAs in both ESCs and NPCs (Figures 2C and D). These results indicate that while FMRP associated microRNAs may target distinct sets of mRNAs which play a critical role in differentiation, FMRP associated snoRNAs may have a more general role affecting overall translation. FMRP associate with a group of C/D box snoRNAs, both in ESCs and NPCs. C/D box snoRNAs are primarily involved in the methylation of specific sites on ribosomal RNAs (rRNAs) through which they facilitate the folding of rRNAs and the assembly of ribosomes. Our preliminary data indicates that absence of FMRP seems to increase the methylation of specific rRNA sites which are targeted by FMRP bound snoRNAs (Figures 2E and F). The significance of FMRP association with snoRNAs is not clear at this stage. However, we propose two possible roles. 1) By associating with specific snoRNAs, FMRP may control the methylation of rRNAs and thus regulate the rate of ribosome biogenesis. 2) Through their association with snoRNAs, FMRP may mark a subset of ribosomes which in turn may be reserved only for the translation of FMRP target mRNAs. We are currently designing experiments to test both of these hypotheses.

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3.3

Mahendra Rao
Raghu Padinjat

ACCELERATOR PROGRAMME FOR DISCOVERY IN BRAIN DISORDERS USING STEM CELLS (ADBS)

This programme uses modern stem cell technology to create cellular models of the brain derived from human subjects taken from families with a history of mental illness. The overall goal is to uncover the genetic, cellular and molecular basis of mental disorders.

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

Mental illnesses are recognised as having an inherited basis. However, despite their high heritability and the identification of a large number of 'common' and rare variants, few genetic correlates that could account for their high prevalence have been identified. Many of the genes (and pathways) identified suggest aberrant neural development and connectivity in early life as being critical to their pathogenesis. The epigenetic changes that occur due to exposure to environmental influences during windows of sensitivity in the developing brain, give rise to different trajectories of brain development lead to variations in temperament, response to stress and substance abuse.

Given the gene/environment interactions that over time are likely to lead to psychiatric disorders, well-defined, prospective clinical cohorts offer a unique opportunity to understand the pathogenesis of mental disorders. In collaboration with the Department of Psychiatry, NIMHANS, we plan to identify a prospective cohort of families with a high density of mental illness. The families will be followed over a period of twenty years in order to observe the development of disease and detailed clinical investigations will be performed at regular intervals. In addition,

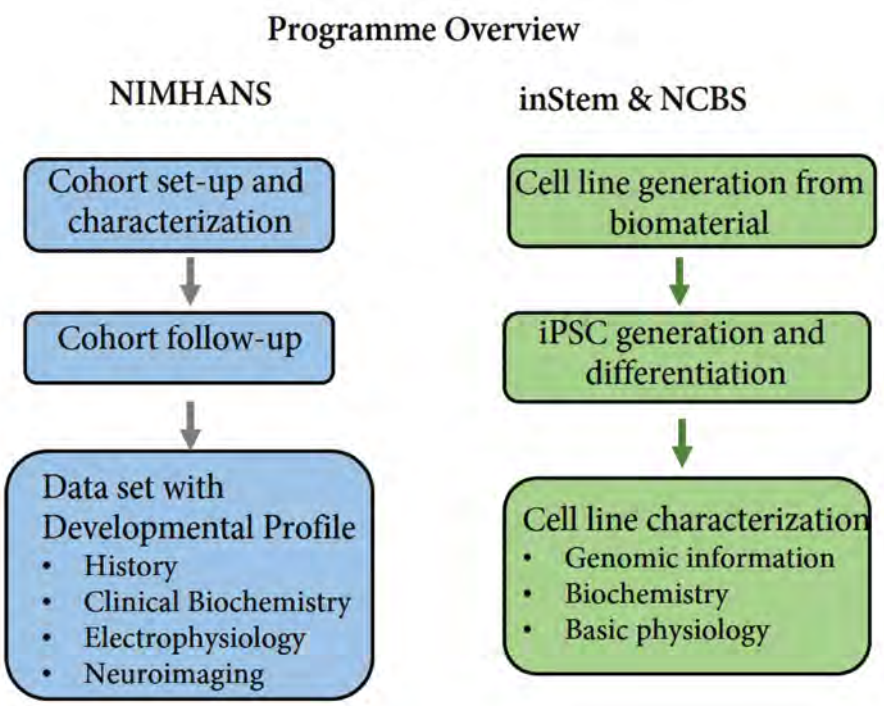

Fig 1

ADBS program is an integrative approach that links basic research with clinical studies of mental illness.

we will establish immortalised stable cell lines and pluripotent stem cells from affected individuals in these families. This material will be used to generate cellular models in which the mechanistic aspects of cellular neurobiology that may lead to disease can be understood.

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

The Accelerator programme for Discovery in Brain disorders using Stem cells (ADBS) is a new scientific venture to understand mental illness by harnessing the power of modern human genetics and stem cell technology. The Program is a collaborative initiative of three institutions from Bengaluru, India – the National Centre for Biological Sciences (NCBS), the Institute for Stem Cell Biology and Regenerative Medicine (inStem) and the National Institute for Mental Health and Neurosciences (NIMHANS). The program receives support from the Department of Biotechnology, Government of India and the Pratiksha Trust.

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3.4 *Archana* Purushotham

NEUROIMAGING AND CLINICAL NEUROSCIENCE

Our lab is interested in the functioning of the human brain in health and disease using neuroimaging. We are also actively researching traditional Indian medicine – Ayurveda – through clinical and epidemiological studies.

Cognition and connectivity

Normal humans differ widely in their cognitive abilities, be it in language, mathematics, music or the emotional intelligence domains. Although this fact is widely recognised, the basis of this variation in normal subjects is not yet understood. We are conducting a research study to examine the neural basis of this variation in adult human subjects.

Existent standardised cognitive tests have mostly been developed for, and standardised on Western populations. We first put together a battery of cognitive tests designed to bring out the variation in cognitive abilities in Indian subjects. This battery covers multiple domains including memory, vocabulary, comprehension, logic, arithmetic, emotional intelligence, music perception, and visual-spatial ability. Using an online version of this battery, we are in the process of gathering normative data for the Indian population.

To study the neural basis of this variation, we use magnetic resonance imaging (MRI) techniques called functional MRI and voxel-based morphometry (VBM). Normal young adult volunteers are administered the above test battery, and their brains are then scanned in a high-field (3 Tesla) scanner. The MR images are analysed to measure the strength of functional connectivity, grey matter volumes and cortical surface areas in different regions of the brain. We will then attempt to correlate the scores on various tests with these connectivity and morphological measures.

Besides looking at neural correlates of performance on individual tests, we are especially interested in determining if there are connectivity or morphological parameters that underlie performance across multiple tests and domains. In particular, higher level control networks, owing to their involvement in multiple cognitive tasks, could potentially affect performance on multiple

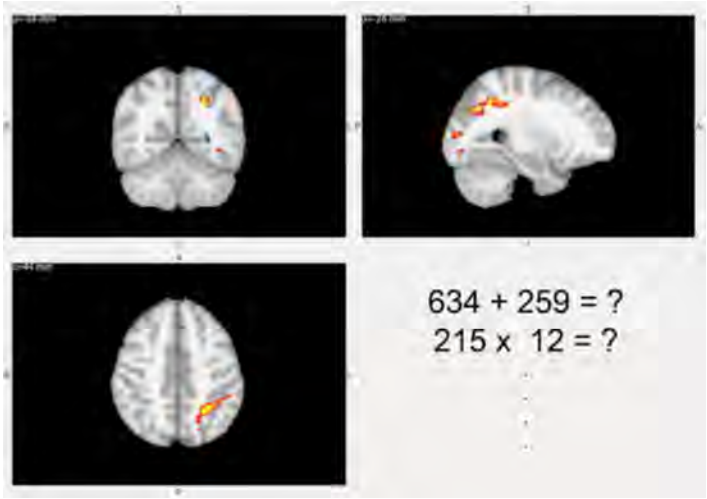


Fig 1

Brain images showing activation in the region of the left intraparietal sulcus in subjects during performance of simple mental arithmetic.

tests. Establishing a relationship between neural network connectivity/cortical morphology and cognitive performance would be a significant step forward in understanding the neural basis of the spectrum of intelligences among people.

Ayurvedic treatment of stroke

Allopathic care for neurological disease is very limited. Hence we are very interested in integrative approaches to care combining Ayurveda with Allopathy. Stroke is the number one cause of major long-term disability the world over, and in South Asia, increased longevity and modern lifestyles have created a stroke "epidemic" in recent years. Yet only a negligible proportion of ischaemic stroke patients in India actually receive any specific treatment for stroke. This is because the only specific and proven Allopathic therapies for ischaemic stroke need to be administered in an intensive care setting within the first few hours after it occurs. Ayurvedic stroke therapy does not have a strict time limit, and if efficacious, may offer an alternative or adjunctive therapy to improve stroke outcomes. We are in the final phase of a pilot study to examine the safety and efficacy of Ayurvedic treatment in ischaemic stroke patients, compared with Allopathy.

Ayurvedic *prakriti* and stroke

Ayurvedic theory divides people into different constitution types, or *prakriti,* based on physical and mental characteristics, and treatment is tailored to the specific *prakriti,* akin to the personalised medicine concept of Allopathy. *Prakriti* is believed to determine susceptibility to disease. We are investigating the validity of this concept. We have developed a *prakriti* analysis tool that can be used by laypeople, and have conducted extensive rounds of testing and modification. We are collecting data using a case-control design, to look at *prakriti* profiles of some common and major diseases. One of the diseases we are focusing on currently is stroke. We will compare *prakriti* profiles of stroke patients with those of controls, and determine if *prakriti* represents a risk factor for stroke.

Invited Talks

"Ayurvedic Constitution type and Disease Susceptibility" at symposium on "History of Psychiatry in India: Traditional Systems and Mental Health" at NIMHANS, Bengaluru, India. 15 – 16th June, 2016.

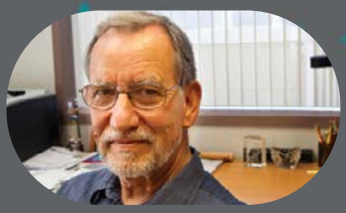
"Stand-Alone Ayurvedic Treatment in Acute Ischaemic Stroke: A Non-Randomised Controlled Trial" at the 21st International Conference on Frontiers in Yoga Research and its Applications, Bengaluru, India. 3 – 7th January, 2016.


Cc

4

Centre for Cardiovascular Biology and Disease

The Centre for Cardiovascular Biology and Disease theme in inStem focuses on the signalling and biomechanical properties of the heart, with an initial emphasis on genetic hypertrophic and dilated cardiomyopathies, and autosomal dominant myocardial diseases caused by missense mutations primarily in one of the several genes encoding the fundamental contractile apparatus of the heart. These diseases are common, debilitating and often lead to sudden death. Our team is composed of seven superb investigators with diverse backgrounds – John Mercer, Minhaj Sirajuddin, R. Sowdhamini, Maneesha Inamdar and Dhandapany Perundurai, based in Bengaluru, and Adjunct InStem faculty Sivaraj Sivaramakrishnan and James Spudich, based in the United States of America. This group brings together a team of scientists using complementary approaches to a fundamental clinical issue in India and worldwide. Interactions and collaborations across our team members are strong, bringing together biochemistry, biophysics, biology, genetics, structural biology, computational biology and clinical sciences to define how cardiomyopathy mutations affect the power output of the human heart. Our ultimate goal is to understand the underlying molecular mechanisms of hypertrophic and dilated cardiomyopathies in order to develop new therapeutic approaches for these diseases.



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Sivaraj sivaramakrishnan

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4.1 *John* Mercer

UNDERSTANDING PRIMARY INHERITED CARDIOMYOPATHIES AT MULTIPLE LEVELS OF ORGANISATION

Mutations in any one of the genes encoding the proteins that produce muscle contraction cause inherited primary cardiomyopathies. Our group's goal is to determine the commonalities and differences in the mechanisms by which these mutations cause disease.

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

Primary cardiomyopathies are disorders of the heart muscle, in the absence of any other disease. They affect 1 in 500 people. The best-described causes are the hundreds of different missense mutations in any one of the genes encoding the cardiac sarcomeric proteins. In response to the primary dysfunction, the morphology of the heart is remodelled, most often along one of two different patterns, with the walls becoming thicker (hypertrophic cardiomyopathy or HCM) or thinner (dilated cardiomyopathy or DCM).

Our group at inStem is trying to understand inherited cardiomyopathies at multiple scales of organisation. We want to determine the precise molecular mechanisms by which a change of a single amino acid in a sarcomeric protein can lead to disease and sudden cardiac death or heart failure. Having studied a set of tropomyosin mutants, we are leveraging what we have learned to study a set of troponin T mutants. Troponin T is a component of the calcium-sensing machinery in muscles, and disease-causing mutations are concentrated in the region of troponin T that

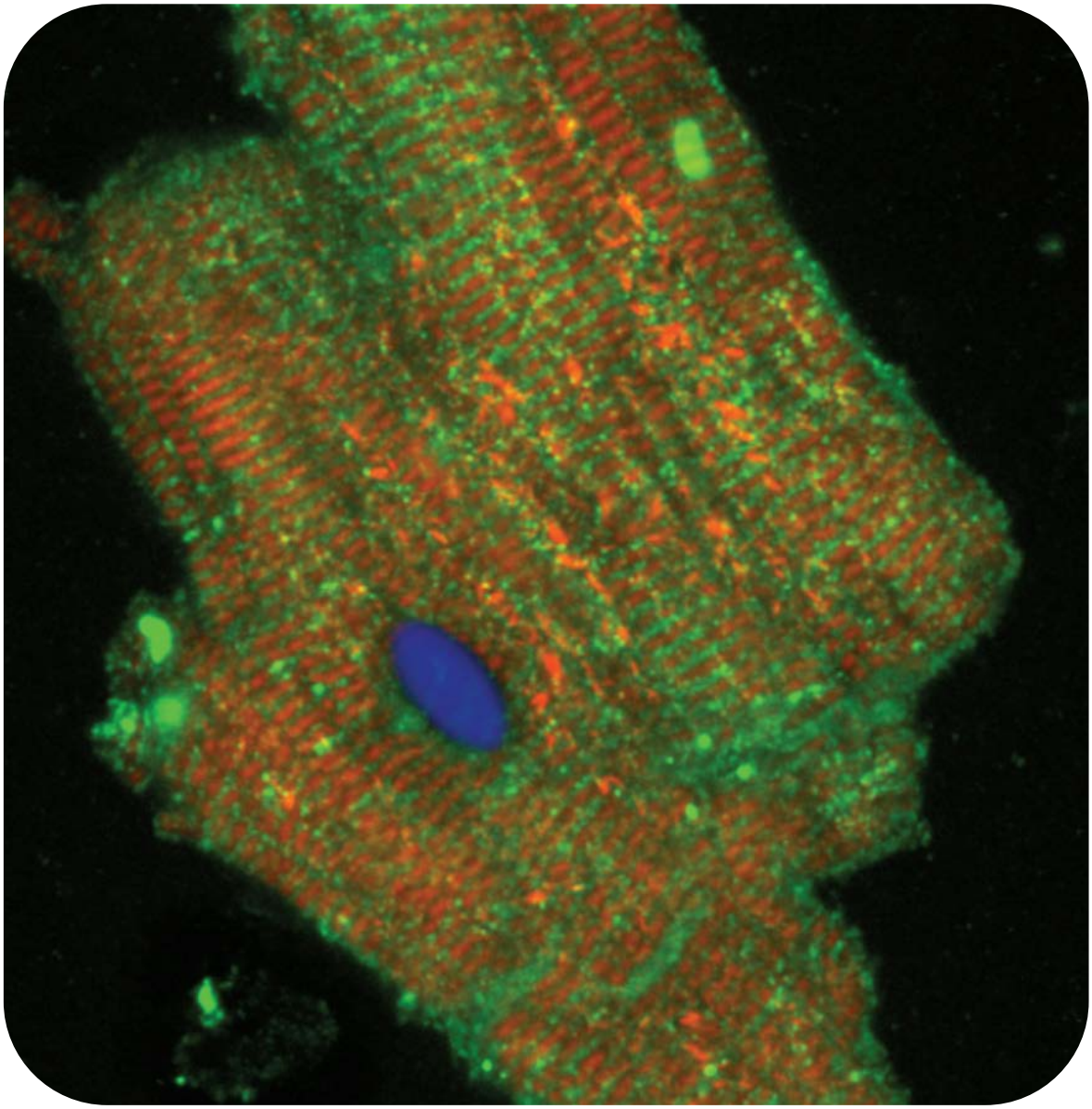

Fig 1

Cardiomyocytes from humanised mouse expressing human myosin, showing actin (red), human myosin (green), and nucleus (blue).

interacts with tropomyosin. We are collaborating with R. Sowdhamini (NCBS), whose group has great expertise in studying these types of protein structures.

We are introducing mutations into human embryonic stem cells (hESC) using the CRISPR/Cas gene editing technology to generate mutant hESC lines in collaboration with Maneesha Inamdar (JNCASR and inStem). The mutant hESCs will be differentiated into cardiomyocytes in collaboration with Kouichi Hasegawa (iCeMS and inStem), to study their force production at the single-cell level. At the organismal level, we are characterising our humanised mouse model, expressing human cardiac myosin in place of mouse cardiac myosin, to study mutations

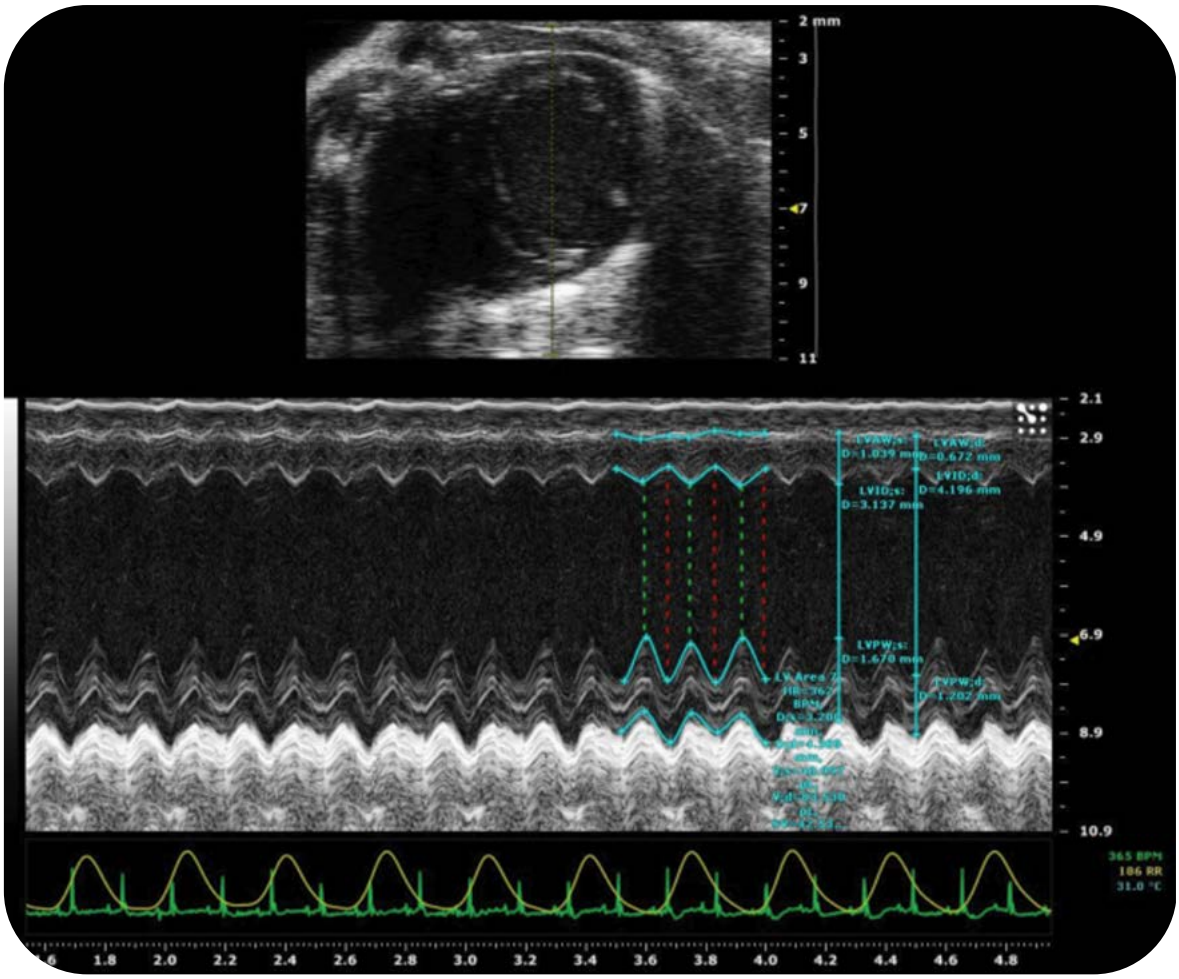

Fig 2

Normal echocardiogram from humanised mouse.

at the level of the whole organism. Despite the very different biochemical properties of the two myosins, mice expressing human myosin are surprisingly normal in their cardiac phenotypes. This characterisation is being performed in collaboration with N. Ravi Sundaresan (IISc)..

Invited Talks

"A multilevel approach to inherited sarcomeric cardiomyopathies" at the International Conference on Cardiovascular Translational Research and the 13th Annual Conference of the International Society for Heart Research (Indian Section) at IIT Madras, Chennai, India. 22nd January 2016.

"A multilevel approach to inherited sarcomeric cardiomyopathies" at the 14th FAOBMB Congress and 84th Annual Meeting of SBC (I), at Hyderabad, India. 30th November 2015.

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4.2 *Minhaj* Sirajuddin

STRUCTURE AND FUNCTION OF CYTOSKELETAL ASSEMBLIES ASSOCIATED WITH DISEASE PATHOLOGIES

Our group is interested in studying how actin and microtubule assemblies coordinate cellular functions at the molecular level. We employ a range of methods to study these cytoskeleton assemblies with implications towards understanding cell physiology and its deregulation in human diseases including, but not limited to cardiomyopathies, neurological diseases and cilliopathies.

As a part of the cardiomyopathy team, my research will focus on bridging the knowledge gap between clinical findings and molecular mechanisms underlying cardiomyopathy disease causing mutations. Our theme has a new member on board, Dr. Dhandapany Perundurai. Together we cover aspects of human genetics, animal models, cell biology, biochemistry and structure at the molecular level of cardiomyopathy diseases. Currently, our group is focusing on reconstituting a minimal contractile unit and crystal structures of sarcomere proteins implicated in cardiomyopathy. Both these projects are in the beginning stages, which will benefit from the collaborative research environment of the CCBD theme and Bangalore Bio-cluster campus.

A major hurdle in achieving reconstitution of a minimal contractile unit is generating arrays of myosin motors that approximate the order found in a half-sarcomere (i.e., one half of a bipolar thick filament). Engineering a defined number of myosin motors and understanding their co-operativity during force generation represents a fundamental challenge in muscle biology and motor biophysics. One of the main goals of our lab is to engineer thick myosin filaments with precise control over the number of myosin heads and their topology. In addition to addressing fundamental questions in the field of muscle biology, the reconstituted system will allow us to study cardiomyopathy mutations and their effects in force generation during muscle contraction. Here, we will utilise the self-assembling DNA origami system to achieve a near native structure myosin thick filaments (dimeric myosin heads displayed in a helical array). This will enable us to study the collective biophysical properties of the contractile unit and compare them with cardiomyopathy disease causing mutations. For designing a synthetic hemi-thick filament

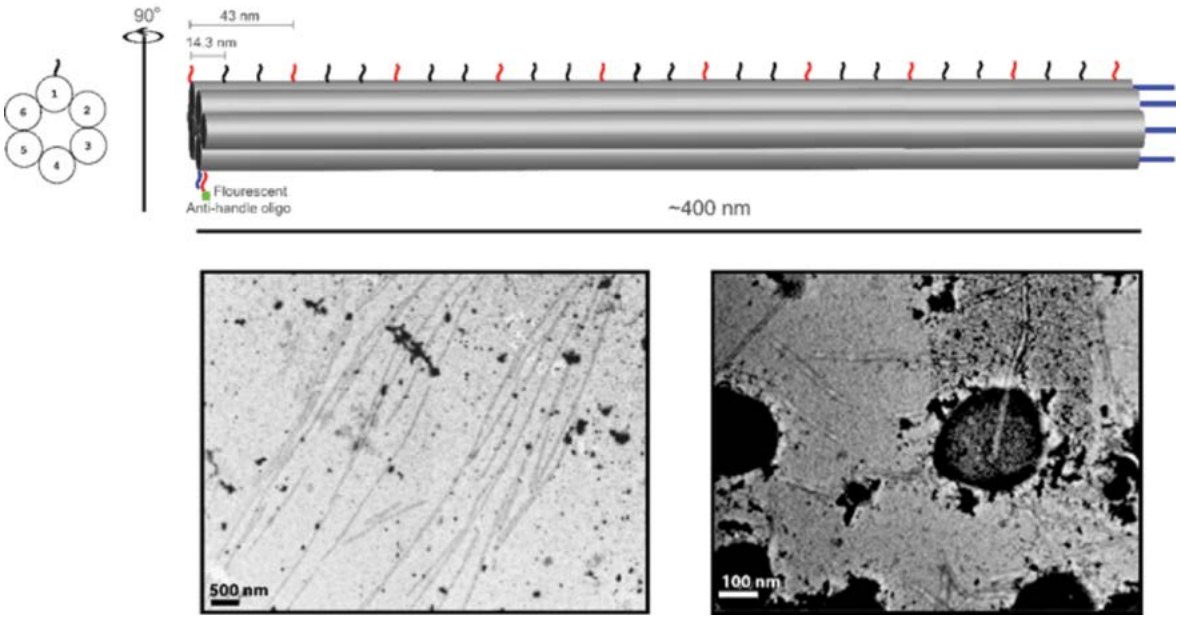

Fig 1

Illustration of DNA origami module design. The spacing of handle sites are similar to native myosin thick filaments (top). Electron micrograph of folded DNA origami structures with right dimensions, 400nm (bottom, left) and aggregated/higher order structures (bottom, right).

assembly we are using computational methods to design a modular assembly to achieve 400 nm wide DNA structure with precise topology of attachment sites for the dimeric myosin heads (Figure 1). We have successfully folded the designed DNA origami structure and validated it using negative stain electron microscopy (Figure 1). Our current efforts are towards optimising the folding of structure to produce homogenous populations of molecules.

Simultaneously we have developed methods to attach SNAP tagged motors to attach to a linear DNA assembly. On the myosin motor front, we have established methods to attach native myosin heads to the DNA. Further steps will involve measuring the processivity and velocity of myosin assemblies. In addition to titrating the number of myosin heads, the DNA origami system allows the possibility of varying motor spacing and adding stalling elements (e.g., rigor myosin). The motility assays planned in the coming months will involve imaging the movement of myosin assemblies along actin filaments using TIRF microscopy.

Several proteins connect the thin and thick filaments and the Z-disc units to each other and mutations in these proteins have also been implicated in cardiomyopathies. For example, titin links thick filaments and the Z-disc; myosin binding protein links thick and thin filaments; and Ð-actinin from the Z-disc forms an anchoring point for thin actin filaments. New evidence points out that the Z-disc not only provides a boundary, but also plays important roles in the stabilisation of sarcomeres, mechano-sensation and signal transduction. There are over a dozen proteins present in Z-disc structures and mutations in every single component lead to a variety of diseases, including HCM and DCM. Among these, nexilin is the least characterised of the proteins that have been implicated in primary cardiomyopathies. So far, clinical studies have identified 5 mutations in nexilin, and the mutations that cause HCM and DCM are clustered at the amino- and carboxy-terminal of nexilin respectively. Nexilin protein in the Z-disc of skeletal and heart muscle

is encoded by the same gene. However, mutations in the nexilin gene cause only cardiomyopathies and no skeletal disorders have been reported so far.

In order to address what biochemical changes occur upon mutation and to understand the different roles nexilin plays in heart and skeletal sarcomere, we aim to undertake structure-function studies of nexilin. Our preliminary results in this direction include progress in cloning and expression of mouse, human and zebra fish full-length nexilin as well as various truncated versions. We have also successfully purified the full-length mouse nexilin and setting up crystallisation trials. On the functional side, we have identified a novel high molecular nexilin isoform in the mouse skeletal muscle; we are currently mapping its amino acid sequence using proteomic approaches. The purified nexilin protein will allow us to identify new interacting partners, and check for F-actin binding and compare wild type versus mutant nexilin proteins.

In addition to the structure-function studies, we are collaborating closely with Dr. Dhandapany Perundurai's lab to identify new mutations in nexilin and other sarcomere proteins. Dr. Perunduria's lab has already identified a novel mutation in nexilin that is present in the Indian population. Future work will involve introducing this mutation in mice to study and model the mutation effects seen in human patients.

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

Publications

McKenney, R.J., Huynh, W., Vale R.D. and **Sirajuddin M.** (2016). Tyrosination of Ð-tubulin controls the initiation of processive dynein-dynactin motility. ***EMBO J*** 35(11):1175-85 (bioRxiv).

Invited Talks

"Regulation of microtubule motors by modifications on their tracks" Max-Planck-Institute, Dortmund, Germany – June 2016.

"Regulation of microtubule motors by modifications on their tracks" at Curie Institute, Orsay, France – June 2016.

5

Centre for Chemical Biology and Therapeutics

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

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Theme Coordinator

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5.1 *Ashok* Venkitaraman

CENTRE FOR CHEMICAL BIOLOGY AND THERAPEUTICS

The Centre for Chemical Biology and Therapeutics (CCBT) pioneers innovative approaches to create chemical tools that modulate novel classes of targets, in order to explore the fundamental biological mechanisms underlying human diseases, with the long-term vision to seed the discovery of new therapeutics.

Selective modulation of intracellular signalling pathways is a major challenge impeding deeper understanding of the biology of human diseases, as well as their therapy using small-molecule drugs. Thus far, efforts in academia and the pharmaceutical industry have focused largely on the inhibition of enzymes such as protein kinases using ATP-competitive inhibitors. These approaches suffer from the lack of chemical and biological selectivity. Chemically, many ATP-competitive inhibitors exhibit off-target binding owing to structural similarities between the ATP-binding catalytic folds of many protein kinases. Biologically, inhibition of proximal catalytic steps in signal transduction can lead to a wide variety of phenotypic effects.

Signal propagation in pathways initiated by enzymes like protein kinases or ubiquitin ligases occurs through the molecular recognition of site-specific post-translational modifications by distinct protein domains. Indeed, multiple structural mechanisms for such recognition have been identified as being critical for signal propagation in diverse pathways that not only mediate fundamental biological processes from bacteria to man, but are also implicated in the pathogenesis of human diseases. Therefore, the creation of small-molecule chemical tools that target the molecular recognition of site-specific post- translational modifications offers a potentially attractive new approach for the selective modulation of intracellular signalling pathways, which could markedly extend the reach of chemical biology and seed therapeutics development.

Our programme aims to explore this concept, with the first objective of creating a palette of selective chemical tools that modulate the recognition of site-specific protein phosphorylation

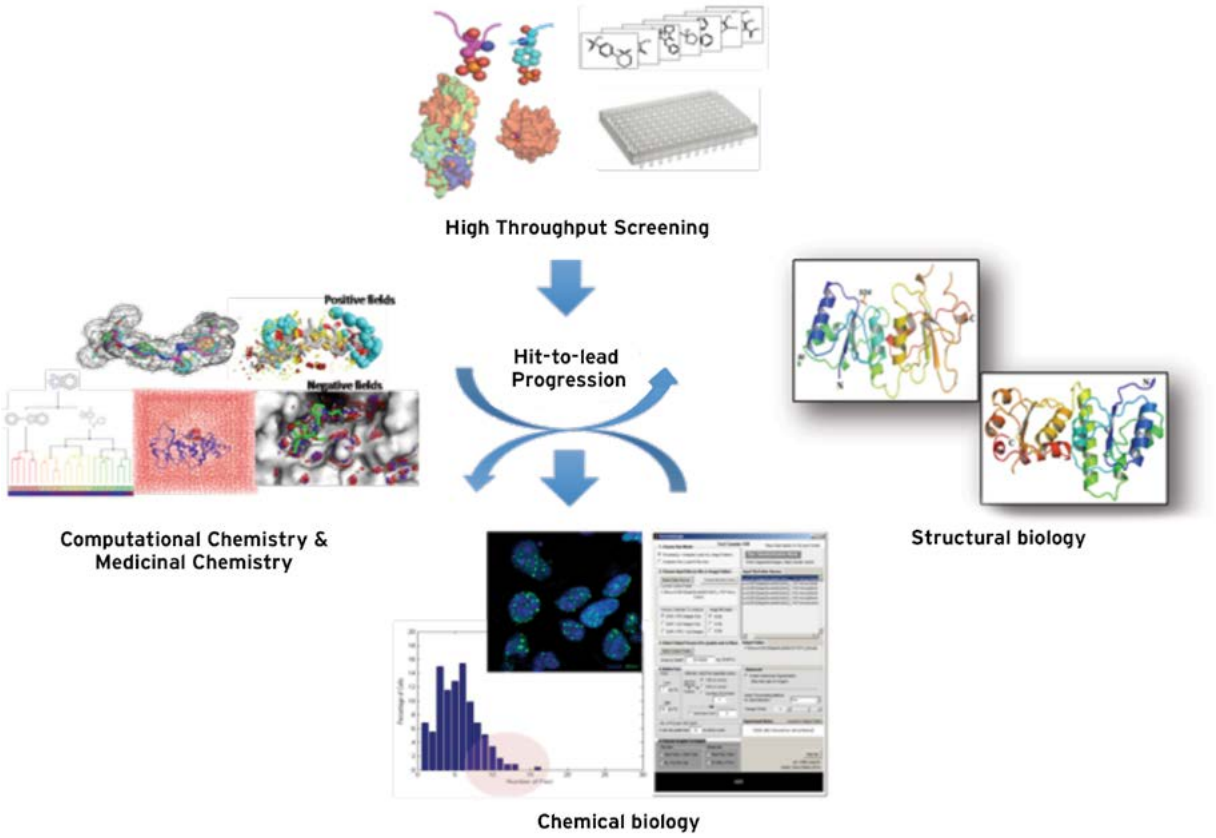

Fig 1

Computational chemistry approaches were used to populate an ~130K element chemical library with diverse ligands, enriched for moieties that may bind protein targets of interest. High-throughput screening (HTS) using biophysical and biochemical assays, has identified chemical ligands that selectively bind to protein targets (top panel). Validated hits from such screens have entered hit-to-lead progression (middle panel), in which iterative cycles of computational chemistry, structure determination of liganded targets using crystallography, and chemical synthesis, will underpin progressive exploration of structure-activity relationships. Progression will create potent, selective chemical compounds suitable for chemical biology studies (bottom), which integrate approaches from molecular cell biology, somatic cell genetics and cellular imaging, to dissect the mechanism and biological consequences of target engagement.

by specific domains. This is a challenging goal, beset with many underlying technical as well as conceptual hurdles. Therefore, our programme takes a stepwise approach that aims to provide deeper scientific insight into the structural mechanism of phospho-site recognition, as well as innovative new approaches to develop chemical tools that selectively target it.

Our programme began in April 2014 with the fitting out of the new CCBT laboratories in the SLC complex at NCBS/inStem, including the installation of major new equipment for high-throughput screening with liquid-handling robotics, protein biochemistry, and biophysical screening. Staff recruitment phased to coordinate with stepwise progression of the first scientific programme was completed in 2015. Team leads for Biochemistry/High-Throughput Screening, Computational Chemistry, Molecular Cell Biology and Structural Biology have been appointed, and have then recruited teams of post-doctoral and technical staff to initiate research in each area. Figure 1 shows an outline of our scientific workflow.

Scientific progress during 2015-16 has rapidly followed the initiation of the CCBT's work. Highlights include the following achievements.

Three hitherto "undrugged" molecular targets representing distinct structural mechanisms for phosphopeptide recognition have been cloned, expressed and purified in milligram quantities. A

focused chemical library comprising ~130K elements has been designed, sourced and organised in an appropriate LIMS system. High-throughput primary screening assays have been optimised for each target in automated assay formats suitable for high-throughput screening, with Z scores ⧺0.6. Over 1.5 million in vitro screening reactions have been completed. Over four hundred active compounds that selectively inhibit molecular recognition by phosphopeptide-recognising domains have been identified by HTS, speaking to the potential to develop target-selective inhibitors against such previously 'undrugged' targets. Validation of the active compounds with dosimetry through orthogonal assays has been completed, yielding >100 chemical ligands encompassing varying potency against different targets of interest.

We have utilised our now-established infrastructure and resources for computational chemistry and synthetic chemistry to investigate the binding mode of different chemical ligands, and explore their structure-activity relationship (SAR). Chemotypes represented amongst the validated ligands have been prioritised for progression based on physicochemical traits, synthetic tractability and particularly, on computational studies using docking and molecular dynamics (MD) simulations to describe potential modes of binding to the target protein. Formulation of binding-mode hypotheses for different ligands has enabled the proposal of analogs for chemical synthesis. Iterative testing of analogs has enabled us to test the binding mode hypotheses, and importantly, to improve the potency of the compounds against different targets of interest. This iterative work (in which over 12 different chemotypes have been analysed, and over 150 new compounds have been synthesised) has recently led to the identification of two chemical clusters exhibiting potencies estimated in the 10-100 nanomolar range against a previously 'undrugged' phospho-peptide recognising domain.

Computational work has also sought to improve the selectivity of chemical ligands identified in this way against other phospho-peptide-recognising domains. We have extensively analysed shape, pocket vs. substrate volume, and undertaken electrostatics comparisons combined with MD simulations, to identify structural features associated with selectivity. These features are being exploited in chemical syntheses to facilitate the development of inhibitors with high target selectivity.

In parallel, we have initiated the X-ray crystallographic analysis of chemical ligands bound to phospho-peptide recognising domains. Over the past 9 months, we have resolved the unliganded apo-structures for different conformers of protein targets at <4.0Å resolution using the NCBS local X-ray source. Screening for crystallisation conditions to solve the structure of these domains bound to chemical ligands is now underway.

Cell biology work has developed a cascade of biological assays suitable for determining target engagement and compound selectivity in the cellular milieu, for targets of interest. Broadly, one element of the assay cascade aims to detect selective target engagement in the cellular milieu using recently developed methods such as cellular thermal shift, Forster resonance energy transfer (FRET) and fluorescence lifetime measurements in cells. A second element of the assay cascade explores alterations in specific cellular phenotypes such as intracellular localisation of the target, or progression through the cell division cycle, to test the biological mechanisms underlying the activity of chemical ligands. Compounds with sufficient potency and selectivity against specific protein targets have recently emerged from chemical synthesis and *in vitro* biochemical analyses, which will be introduced into cellular assay cascades.

The CCBT Team

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Gayathri Sadasivam
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Kavitha Bharatham
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Aneesh Goyal
Team Lead,
Structural Biology

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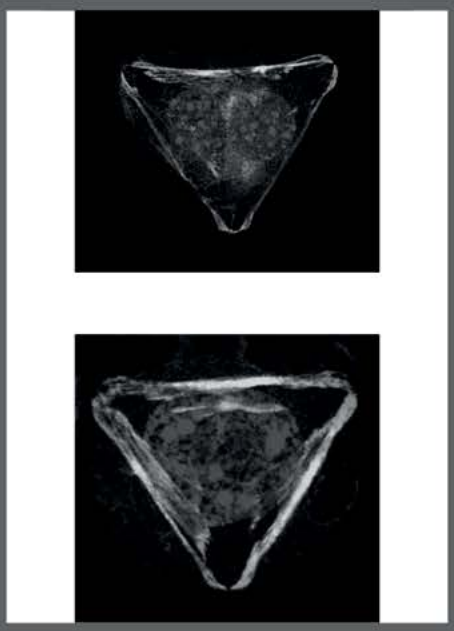
6

Centre for Inflammation and Tissue Homeostasis



The Centre for Inflammation and Tissue Homeostasis (CITH) seeks to make the promise of regenerative medicine a reality and replace/repair tissues lost to disease, trauma, or aging. Currently the theme is comprised of three faculty members Shravanti Rampalli, Srikala Raghavan, Colin Jamora, a research technologist Subhashini Sadasivamand a visiting professor from the Genome Institute of Singapore, Ramanuj DasGupta. Research is centred around two foci: 1. The regulation of cells that mediate tissue regeneration and repair; 2. The study of epithelial homeostasis and immune regulation at barrier surfaces. Although these studies utilise multiple experimental platforms, a common model system that is studied is skin homeostasis, regeneration, and repair.

Over the past year, work emerging from CITH has provided important new insights into the mechanisms regulating a sterile inflammatory response. This dovetails nicely with the burgeoning interest in CITH of cancer stem cells and the tumour stroma, which is marked by inflammation that drives tumourigenesis and metastasis. Likewise, the discovery of unexpected connections between immune cells and resident cells of the skin have opened exciting new avenues of research on the impact of immune cell function on skin anatomy and its regenerative capabilities. Moreover, novel mechanisms have been identified for histone methyltransferase in governing epigenome organisation and nuclear architecture that have fundamental implications in understanding the natural progression of aging and disease in human cells.

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6.1 *Colin* Jamora

MECHANISMS REGULATING TISSUE REPAIR AND REGENERATION

The IFOM-inStem joint research laboratory works on deciphering the molecular mechanisms underlying wound healing. The goal is to utilise this knowledge to develop therapies for diseases where wound healing is deregulated, such as in diabetes, fibrotic diseases and cancer.

The Jamora lab operates under the auspices of a research agreement between the FIRC Institute for Molecular Oncology (IFOM) in Milan, Italy and inStem. Our research is focused on understanding the mechanisms underlying wound healing, and tissue homeostasis. The process of wound healing is often found to be impaired in many systemic diseases. In diabetic patients, for instance, tissue loss is observed due to the inability to heal, whereas in patients suffering from fibrosis of the skin, liver or kidney, excess scar tissue formation compromises organ function. Moreover, tumour progression reproduces many hallmarks of the wound microenvironment and thus our programme has seamless connections with IFOM, an institute that focuses on the molecular mechanisms underlying tumour formation and development. Our goal is to understand the molecular and cellular crosstalk in normal wound healing and how it is perturbed in pathological conditions, thereby revealing potential avenues of therapeutic intervention.

Various projects are underway to understand the extensive intercellular communication and signalling pathways that mediate the inflammatory, proliferative and remodelling phases of the wound healing response. Two of these will be highlighted to provide a flavor of the ongoing work in the IFOM-inStem Joint Research Laboratory:

1) A major question in the field of wound healing is how the cells immediately surrounding the wound sense that an injury has occurred and launch the complex processes required for tissue repair. This is an important issue as many diseases with a so-called "wound signature", such as eczema, psoriasis, scleroderma, fibrosis, and cancer are essentially a chronic wound

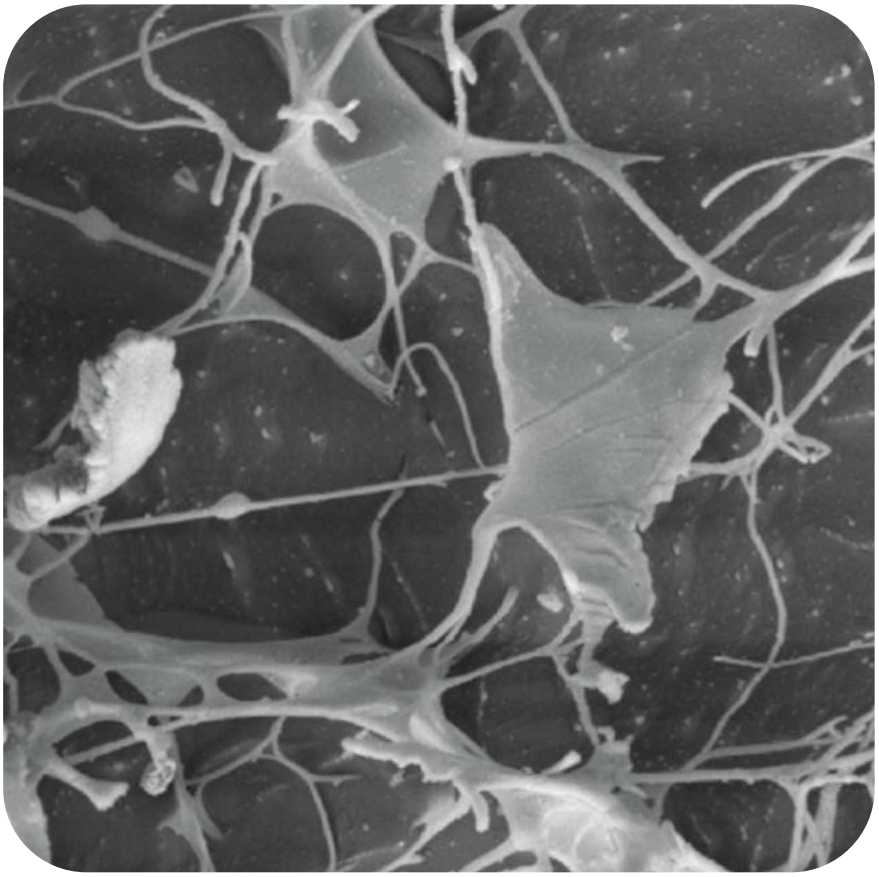

Fig 1

Scanning EM of primary dermal fibroblasts induced to form nanotubes when grown in "wound-like" conditions. Bulges in the tubes are cargo in the process of being transferred from one cell to the other.

response. Understanding how this response is activated will likely provide insights on how to terminate it. Taking an interdisciplinary approach with collaborations both within India and internationally, we have made significant headway in understanding these wound sensing mechanisms. We have found that epigenetic modes to silence genes is an essential component of this injury sensing apparatus. Within minutes of applying a wound, the translocation from the cytoplasm to the nucleus of an enzyme responsible for DNA methylation is observed. This de novo methyltransferase enzyme (DNMT3) can then methylate and silence a cohort of genes that impede the wound healing response. Interestingly, it appears that this nuclear translocation of DNMT3 is due to the release of cellular tension within the tissue that immediately occurs as a result of a mechanical perturbation that disrupts intercellular adhesion. These results point to an unpredicted convergence of mechanical and epigenetic signals to initiate the repair process following trauma to a tissue.

2) Another research project addresses a relatively new mode of direct intercellular communication within a tissue. In various disease scenarios, it has been shown that cells can exchange cellular cargos such as organelles and proteins through structures known as tunnelling nanotubes (TNTs). These TNTs are actin-based extensions from the surface of the cell that are nanoscale in diameter, and unlike other membrane protrusions such as filopodia and lamellopodia, do not adhere to the substrate. These structures have been garnering increasing attention as they appear to form a conduit between the cells within the tumour stroma and the cancer cells. Given that the tumour stroma has been demonstrated to assist cancer cells from circumventing the effects of chemotherapy, how these different cells communicate with each other is a critical

factor in developing effective anti-cancer therapies. The bulk of the work thus far has been focused on the role of these TNTs in diseases such as cancer, but surprisingly little is known of their biogenesis and maintenance.

We have now shown, using a mouse model of wound healing, that these TNTs can be generated within the skin and link different cells to each other, including fibroblasts in the dermis to each other (Figure 1) and possibly dermal fibroblasts with hair follicle stem cells. Thus the TNTs found in cancer appear to be yet another example of how cancers usurp processes that normally occur in wound healing in order to promote tumourigenesis. Furthermore, we have been able to recapitulate the formation of TNTs *in vitro* and the success of this assay was contingent upon reconstituting the appropriate 3-dimensional microenvironment of the dermal fibroblasts and treating them with the secretome of "wounded" cells. We are therefore now poised to fill important gaps in our understanding of TNT biogenesis such as the identity of extracellular signals that induce the formation of these structures as well as the intracellular pathways that translate these external cues into the development of these cytoplasmic extensions.

Publications

Nakasaki, M., Hwang, Y., Xie, Y., Kataria, S., Gund, R., Hajam, E.Y., Samuel, R., George, R., Danda, D., M.J., P., Nakamura, T., Shen, Z., Briggs, S., Varghese, S. and **Jamora, C.** (2015). The matrix protein Fibulin-5 is at the interface of tissue stiffness and inflammation in fibrosis. ***Nature Communications.*** 6, 8574.

Lee, D.J., Du, F., Chen, S.W., Nakasaki, M., Rana, I., Shih, V.F.S., Hoffmann, A. and **Jamora, C.** (2015). Regulation and Function of the Caspase-1 in an Inflammatory Microenvironment. ***J. Investigative Dermatology.*** 135(8): 2012-2020.

Invited Talks

"Best practices in PhD supervision" at the Manipal University Research Colloquium, Manipal University, India. 2016.

"Elucidating the signals that orchestrate tissue repair and promote disease" at the Centre for Biosystems Science and Engineering Annual Symposium at the Indian Institute of Science, Bengaluru, India. 2016.

"The biochemical and epigenetic regulation of caspase-8 in wound-healing and cancer" at the Physics of Cancer Symposium at University of Leipzig, Germany. 2015.

"Epigenetic regulation of wound healing" at the Kyoto-IFOM International Symposium at Kyoto University, Kyoto, Japan. 2015.

"Role of Snail and Mindin in Tissue Fibrosis" at the Kyoto Graduate School of Medicine, Department of Dermatology, Kyoto, Japan. 2015.

"Regulation of the wound healing response" at the IFOM-MBI Joint Retreat, Sardinia, Italy. 2015.

"Regulation of the inflammatory response during wound healing" at Unilever, Bengaluru, India. 2015.

"Unravelling the intercellular signalling networks that lead to tumour stroma formation" at the ACTREC International Symposium: Molecular Pathways to Therapeutics: Paradigms and Challenges in Oncology, Mumbai, India. 2015.

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6.2 *Shravanti Ramapalli* Deshpande

HISTONE METHYLTRANSFERASES GUIDING DEVELOPMENT AND AGING

Broad areas of research interest in the laboratory lie at the interface of epigenetics and stem cell biology. We are addressing the role of repressive histone methyltransferases in the commitment of cell fate from stem cell through aging.

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

Due to constant renewal and high propensity for repair, the skin along with the gut and the haematopoietic system, is a tissue of choice to explore stem cell biology. Although signalling pathways that regulate skin biology are very well understood, the molecular and epigenetic basis remains largely uninvestigated. Studies from a few labs have indicated the role of DNA methylation and chromatin modifications in skin development, homeostasis and repair. However the epigenetic regulation of skin regeneration is still in its infancy. Therefore dissecting epigenetic mechanisms regulating the epidermal and dermal compartments of the skin will not only shed light on skin development & homeostasis but also will provide insights for aging and repair.

Preclinical evidence suggests that targeting HMTs is beneficial in many types of cancers. While wound healing and cancers are known to share several commonalities, there is a single report demonstrating the reduction of histone methyl marks such as H3K27me3 via downregulation of PRC2 complex in the context of wound healing. To gain functional and mechanistic insights of how

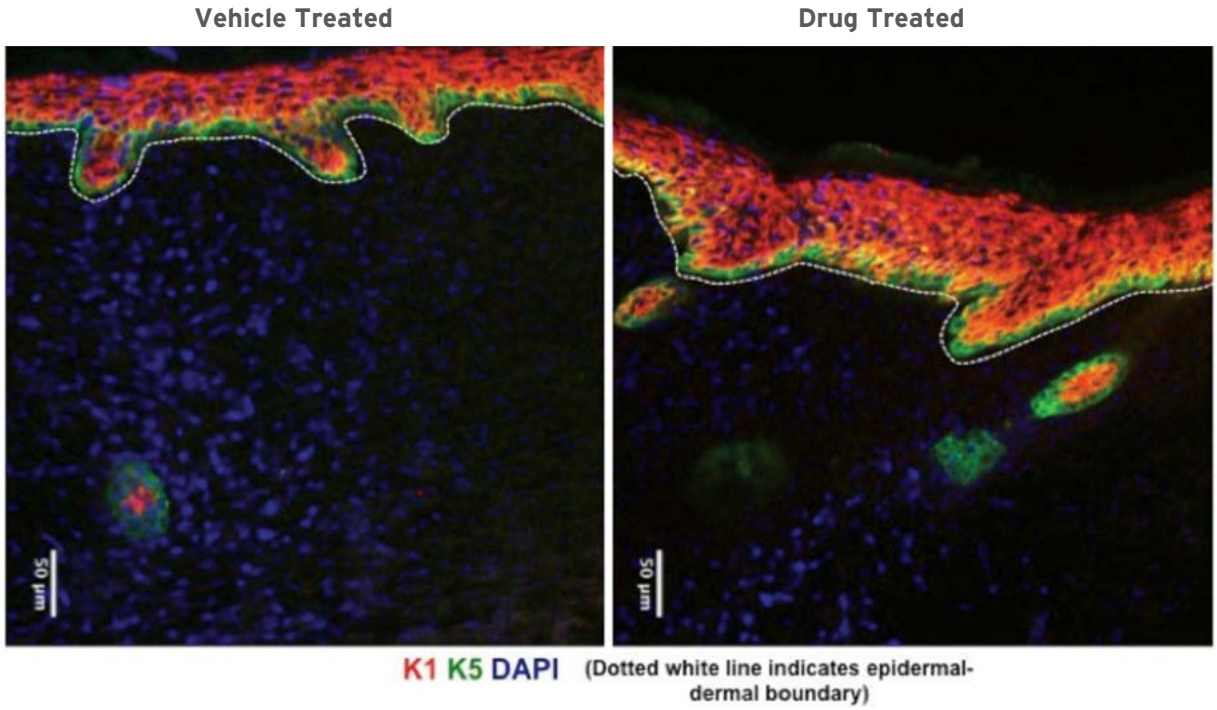

Fig 1

Inhibition of PRC2 activity results in hyper thickened epidermis and impaired wound healing in mice.

methyl marks dictate the outcome of skin tissue repair, we are using small molecule inhibitors targeted against methyltransferase activity in *in vitro* and *in vivo* wound healing assays. This programme will advance our insights into the molecular basis of wound healing and will have direct impacts on our understanding of pathologies in impaired tissue repair upon aging and in diabetic condition.

Lineage restriction is the key property of the differentiated cells. However studies in lower organisms have demonstrated developmental plasticity of fibroblasts to more than one lineage during wound healing/regeneration. Alongside, the last decade witnessed a majority of studies demonstrating the potential of mammalian fibroblasts to dedifferentiate into pluripotent cells and trans-differentiate into a variety of lineages *in vitro*. To understand the mechanistic details of the epigenetic basis of lineage restriction and plasticity of fibroblasts, we studied the role of EZh2 in conversion of fibroblasts towards pluripotent state. Our study demonstrated that Ezh2 facilitates the conversion of human fibroblasts into pluripotent state by overcoming initiation blockade events such as mesenchymal to epithelial transition and cellular senescence. Additionally, our findings provided a mechanistic understanding by which Ezh2 restricts the somatic programme during early phase of cellular reprogramming and establish the importance of Ezh2 dependent H3K27me3 activity in transcriptional and miRNA modulation during human iPSC generation.

In addition to histone methyltransferase activity, HMTs also methylate non-histone substrates. Thus, in a parallel study, we performed proteomics analyses to identify novel interactors of histone methyltransferase that might influence cell fate decisions. Currently, we are dissecting unanticipated associations of non-histone substrates with methyltransferases in the process of dedifferentiation and aging of fibroblasts. Our unique programme on understanding the crosstalk between histone modifiers and non-histone proteins will be instrumental in unravelling the complexity of the rules that guide cell fate decisions.

Publications

Rao, R.A., Dhele, N., Cheemadan, S., Ketkar, A., Jayandharan, G.R., Palakodeti, D. and **Rampalli, S.** (2015). Ezh2 mediated H3K27me3 activity facilitates somatic transition during human pluripotent reprogramming. ***Sci. Rep.*** 5, 8229.

Kaur, R., Aiken, C., Morrison, L.C., Rao, R., Del Bigio, M.R., **Rampalli, S.** and Werbowetski-Ogilvie, T. (2015). OTX2 exhibits cell-context-dependent effects on cellular and molecular properties of human embryonic neural precursors and medulloblastoma cells. ***Dis. Model. Mech.*** 8, 1295–1309.

Invited Talks

"Epigenetic regulation of somatic stem cell plasticity" at the IFOM-inStem Conference on Tissue Homeostasis. 3 – 5th February, 2016; inStem; Bengaluru.

"Non-histone interaction of Ehmt1 regulated cellular aging" at the Indian Society for Developmental biologist (InSDB). 15 – 18th July, 2015; CCMB; Hyderabad.

"Role of Ezh2 in pluripotent reprogramming" at the 5th Chromatin Asia Meeting.15 – 18th January, 2015; JNCASR, Bengaluru.

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6.3 *Srikala* Raghavan

EPITHELIAL HOMEOSTASIS AND INFLAMMATION: INTEGRINS AND SMALL RNAS

Research in the raghavan lab focuses on understanding the role of integrins, its associated proteins and small rnas in maintaining the stem cell niche and extracellular matrix organisation, both of which are critical for the maintenance of epithelial homeostasis.

1. Sterile Inflammation in embryos and its role in reorganising the ECM

The knockout of integrin β1 in the epidermis results in a complete loss in organisation of the epithelial basement membrane (BM), more severe than that observed through the loss of individual α subunit integrin knockouts, resulting in neonatal lethality. Recent work from our lab suggests that this loss of organisation is exacerbated by an early embryonic inflammatory response involving the recruitment of tissue-resident and monocyte-derived macrophages to the dermal-epidermal junction (DEJ), associated with increased matrix-metalloproteinase (MMP)

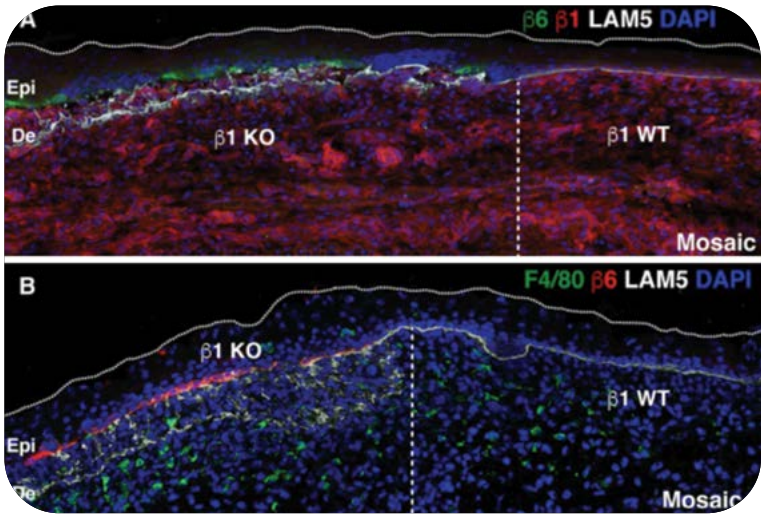


Fig 1

Analysis of mosaic back skin shows that there is a correlation between loss of *β1*, expression of *β6* and recruitment of immune cells. Cells that express *β6* are associated with reduced ECM organisation.

activity. This immune response at the DEJ was found to be driven by the damage associated molecular pattern DAMP Tenascin-C production in proximal dermal fibroblasts and mediated by *de novo* integrin β6 expression in basal epidermal cells. Using novel *in vivo* drug administration experiments, we show the inflammatory response to be defined by classical prostaglandin production and be dependent on paracrine TGF-β signalling. Our data clarifies some of the earliest events during prenatal sterile immune responses, and reveals an important role played by integrin β1 in embryonic skin remodelling.

2. Role of Integrins, Vinculin and RNA binding proteins in regulating cell adhesion, migration and epidermal homeostasis

A) UNDERSTANDING THE ROLE OF VINCULIN IN MAINTAINING STEM CELL HOMEOSTASIS IN SKIN

Vinculin is a mechano-coupling protein found both at focal adhesions and adherens junctions where it contributes to the determination of the mechanical properties of the junctions, links actin cytoskeleton to the junctions, acts as a docking protein for several partners and regulates many signalling pathway transducing mechanical signals. In order to study the roles of vinculin in keratinocytes, we generated a skin specific conditional KO. Detailed analysis of the KO revealed that these animals display defects in the hair follicle cycle (wherein the KO animals had an accelerated hair cycle) while the epidermal development was completely normal. Label retaining experiments performed by pulsing with BrDu and chasing for several weeks revealed that the bulge stem cells fail to maintain their quiescence in the KO, which may explain the continuous cycling of the hair follicles. Thus, the question we are trying to address is, how does the loss of vinculin, a mechano-transducer result in loss of stem cell quiescence? And what role if any, the stem cell niche may play in this? This study will help understand the underlying mechanism that affects the normal hair follicle cycle and the signalling required to maintain the quiescence of hair follicle stem cells.

B) ROLE OF RNA BINDING PROTEINS IN REGULATING CELL-MATRIX ADHESION

Focal adhesions are the major cell adhesion sites between cells and the substratum and at last count contained at least 180 proteins. We identified several RNA binding proteins including

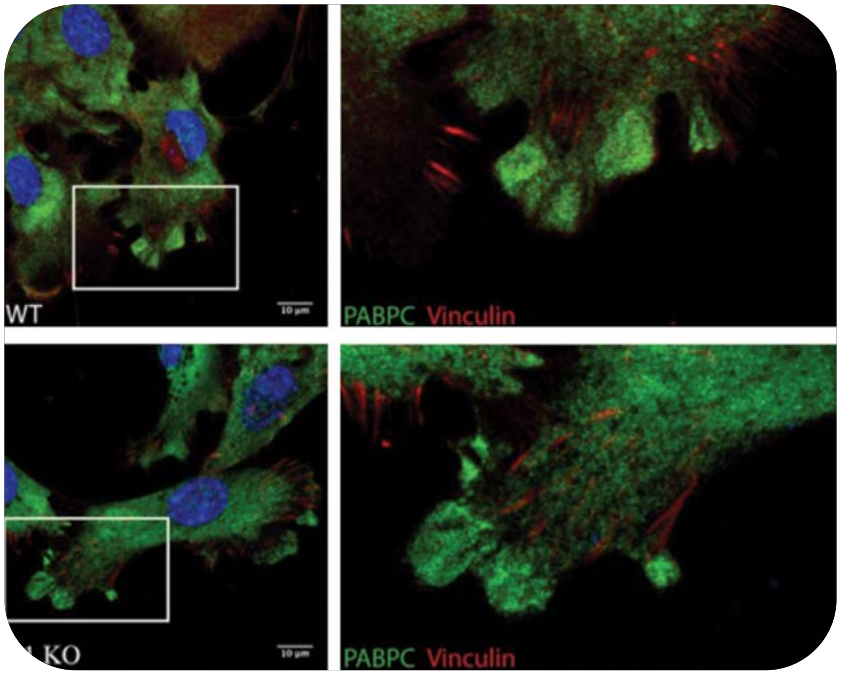


Fig 2

Figure 2: Expression of PAPBC in WT and β1 KO keratinocytes. (PABPC-green, Vinculin-red)

PABPC1, HNPNPs, FUS and DDX that also localise to focal adhesions. The targeting of mRNAs and associated RNA binding proteins has recently emerged as a possible mechanism to generate localised translation at sites where the protein is required.

Our interest in these RNA binding proteins was reinforced when we started collaborating with Dr. Dasaradhi Palakodeti to analyse the phenotypes of PABPC2 knock down in *Planaria* flatworms. The knockdown worms develop epithelial lesions, and upon closer examination, we found that the epithelial layer is no longer associated with the underlying muscle layer due to the loss of organisation of the basement membrane. This phenotype was very reminiscent of the β1 KO phenotype in skin. We have set up a collaboration to look at the role of PABPC1 in keratinocytes and epidermis and the role that it may play in regulating ECM organisation.

The aim of our project is to understand the role of PABPC in epithelial cells, particularly in the dynamics of cell-matrix adhesions and its implication in the regeneration of epithelial tissue, using mouse keratinocytes as a model for in vitro studies and mouse skin in addition to planaria models for *in vivo* studies.

3. Regulation of skin stem cells by small RNAs

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

Publications

Kurbet, A.S., Hegde,S., Bhattacharya, O., Marepally,S., Vemula,P. and **Raghavan, S.** (2016). Sterile inflammation enhances ECM degradation in integrin β1 KO embryonic skin. ***Cell Reports.***

Invited Talks

"Breaking Barriers: Role of Intergins in Epithelial Homeostasis and Sterile Inflammation", Singapore Immunology Network, SigN, Singapore. May 2016.

"Breaking Barriers: Role of Intergins in Epithelial Homeostasis and Sterile Inflammation", inStem Annual Talks, Bengaluru, India. March 2016.

"Breaking Barriers: Role of Intergins in Epithelial Homeostasis and Sterile Inflammation", IFOM-inStem Conference on Inflammation and Tissue Homeostasis, Bengaluru, India. February 2016.

"Skin: The Final Frontier", INK Salon, NCBS, inStem, Bengaluru, India. December 2015.

"Breaking Barriers: Role of Intergins in Epithelial Homeostasis and Sterile Inflammation", Max Planck Institute for Biology of Ageing, Cologne, Germany. September 2015.

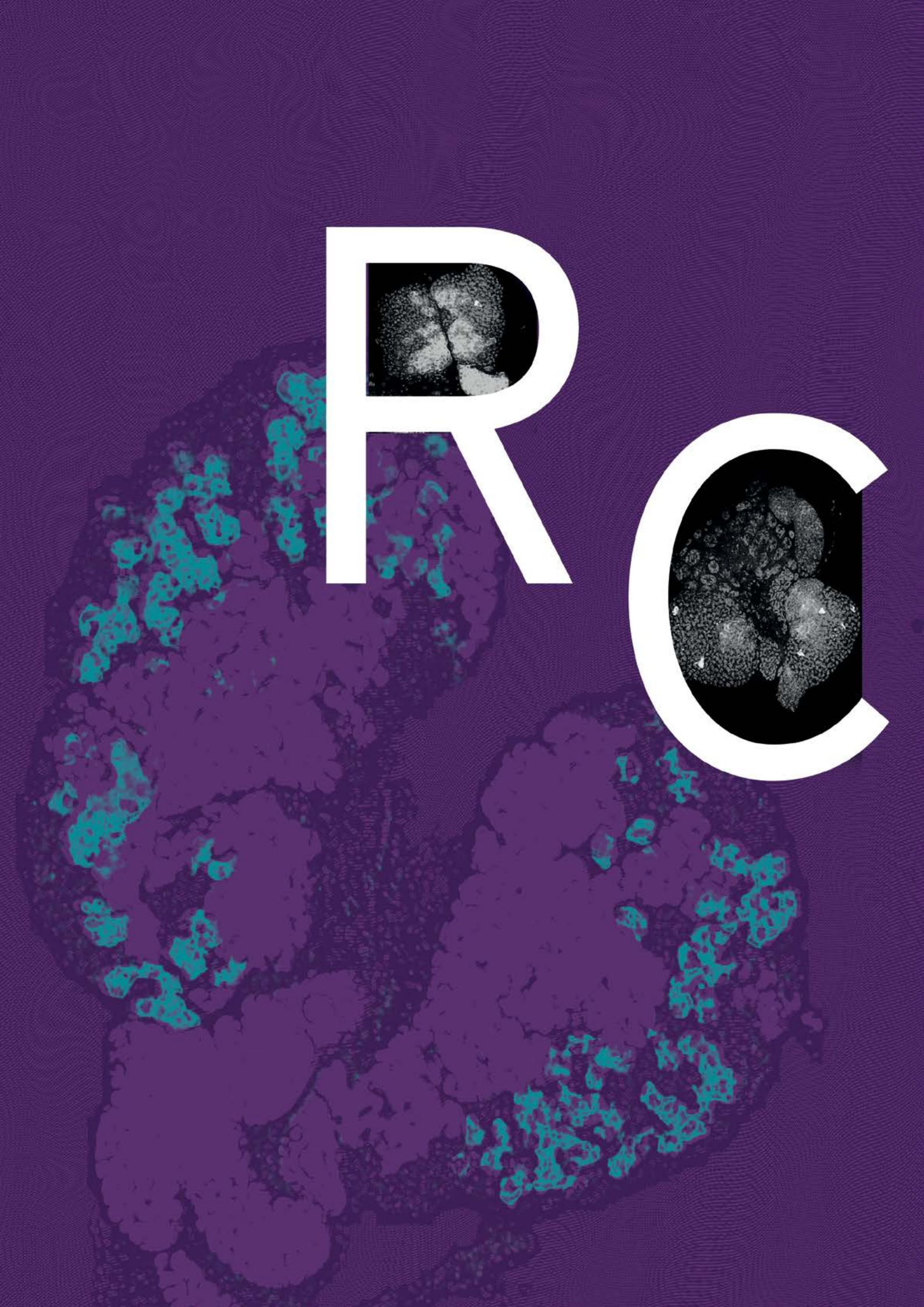
"Breaking Barriers: Role of Intergins in Epithelial Homeostasis and Sterile Inflammation", Max Planck Institute for Biochemistry, Munich, Germany. September 2015.

"Breaking Barriers: Role of Intergins in Epithelial Homeostasis and Sterile Inflammation", Physics of Cancer Symposium, Leipzig, Germany. September 2015.

"Breaking Barriers: Role of Intergins in Epithelial Homeostasis and Sterile Inflammation", IGIB, New Delhi, India. August 2015.

"Breaking Barriers: Role of Intergins in Epithelial Homeostasis and Sterile Inflammation", Yale University, New Haven, USA. July 2015.

"Breaking Barriers: Role of Intergins in Epithelial Homeostasis and Sterile Inflammation", IFOM-MBI Joint Meeting, Sardinia, Italy. July 2015.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Regulation of Cell Fate

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

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Theme Coordinator

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7.1 *Apurva* Sarin

METABOLIC CONTROL OF CELL FATE: NOTCH INTEGRATION WITH METABOLIC PROGRAMMING IN THE T-CELL LINEAGE.

Metabolic reprogramming in t-cells (a cell type in the mammalian immune response) is intertwined with decisions of development, differentiation and homeostasis. Metabolic plasticity is necessitated by needs of the differentiated t-cells and the changing niches they function in. In this context, our research focuses on how nutrients govern transitions critical for t-cell function.

Recycling to renew: Notch integrates with autophagy to promote cell survival

Tuning function in response to nutrient availability is an evolutionarily ancient cellular response. In T-cells (a cell type in the mammalian immune system), the response to nutritional stress constitutes an important cell fate decision, requires metabolic reprogramming and has important consequences for immune homeostasis. Receptor-ligand interactions at the cell surface, operating at the level of single cells or tissues, connect cells to their immediate surroundings and more distal long-range cues. How inputs from receptors are linked to intracellular networks to elicit (appropriate) cellular responses continues to be an area of active investigation. Summarised in this report, is a recently identified interaction of a cell-surface receptor with cellular nutrient sensing and response machinery that regulates cell survival and consequently function.

Nutrient availability enables cells to perform different functions. This can be particularly difficult in the immune system, as key players are mobile and move between regions of plentiful nutrients and sites of injury and inflammation where these might be limited. We are broadly interested in understanding how immune cells adapt to survive and function in these conditions. In this report we describe our experiments with T-regulatory cells, whose primary function is to dampen excess or harmful immune activity, restoring balance in immune function. We built on a previous finding (*Perumalsamy* et al., 2012) of a new role for a well-known pathway mediated by the Notch

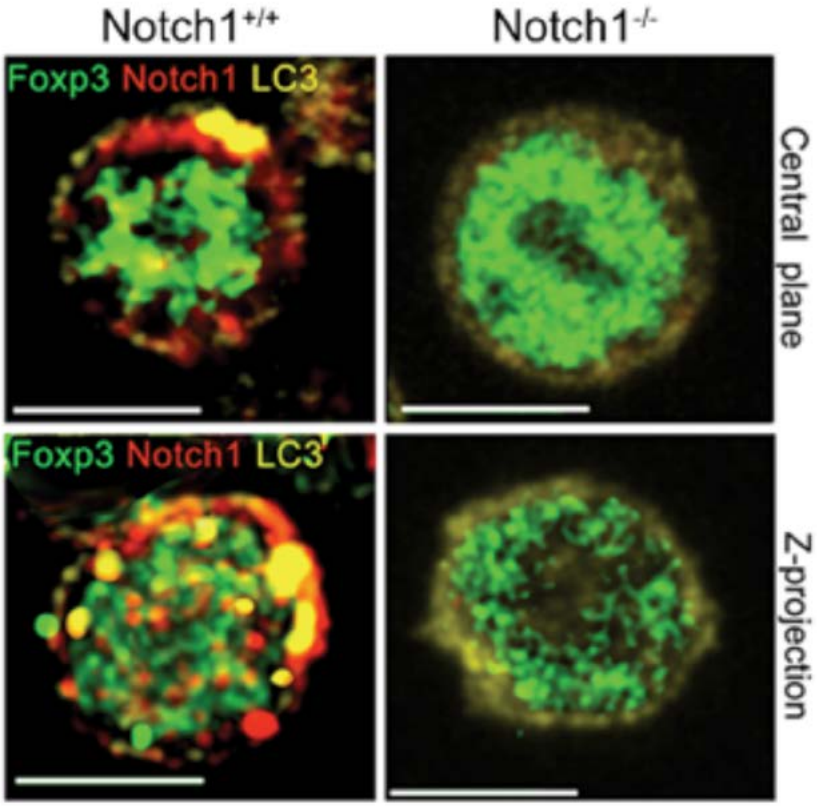


Fig 1

Notch1 regulates activation of autophagy in T-regulatory cells.

Pseudo-coloured, confocal resolution, images of cells stained for Notch1 (red), LC3II puncta (signature of autophagy, yellow) and Foxp3 (marker for T-regulatory cells, green). Despite experiencing the same conditions (nutrient deprivation), the signature of autophagy is not seen in Notch1 deficient (Notch1$^{-/-}$) cells.

Scale bar: 5μm

receptor, a key cell-fate determinant with evolutionarily conserved functions and signalling intermediates. We had shown in earlier work, that the specific activation of Notch and its spatial localisation in T-regulatory cells was key to survival in conditions when nutrients were limited. The work reported here summarises our efforts at elucidating how Notch might bring about this outcome.

Switching T-regulatory cells to nutrient limiting conditions, triggers the activation of a self-contained process of degradation called autophagy (literally translated as self-eating). This well-characterised process, mediated by a spatially defined and regulated protein network, rids cells of molecules and structures that are damaged, regenerating building blocks for survival. Our experiments show that Notch activity correctly times the activation of autophagy (Figure 1). We investigated underlying mechanisms and the functional consequences of this interaction. In conditions that mimic nutrient withdrawal, processed Notch1 receptor generated by interaction with ligand signals from a non-nuclear location and forms a complex with specific molecular intermediates of the autophagy cascade. Disruption of any of the aforementioned events was detrimental to cell survival. A requirement for autophagy for the functional maturation of T-regulatory cells has been recently reported (Wei et al., 2016). Our experiments confirm this observation and reveal that activation of autophagy is under the control of Notch1 and enables T-regulatory cell function. Thus, perturbations of Notch1 in T-regulatory cells, presented with phenotypes consistent with immune inflammation and dysregulated homeostasis (Marcel & Sarin, 2016).

We anticipate that the interactions between Notch and autophagy will be more widely prevalent, possibly tuning cell-fate decisions governed by the Notch receptor in other cell types. Towards this aim we are testing if interactions are conserved between these two evolutionarily ancient and conserved cascades in other contexts. More direct questions arising from this work,

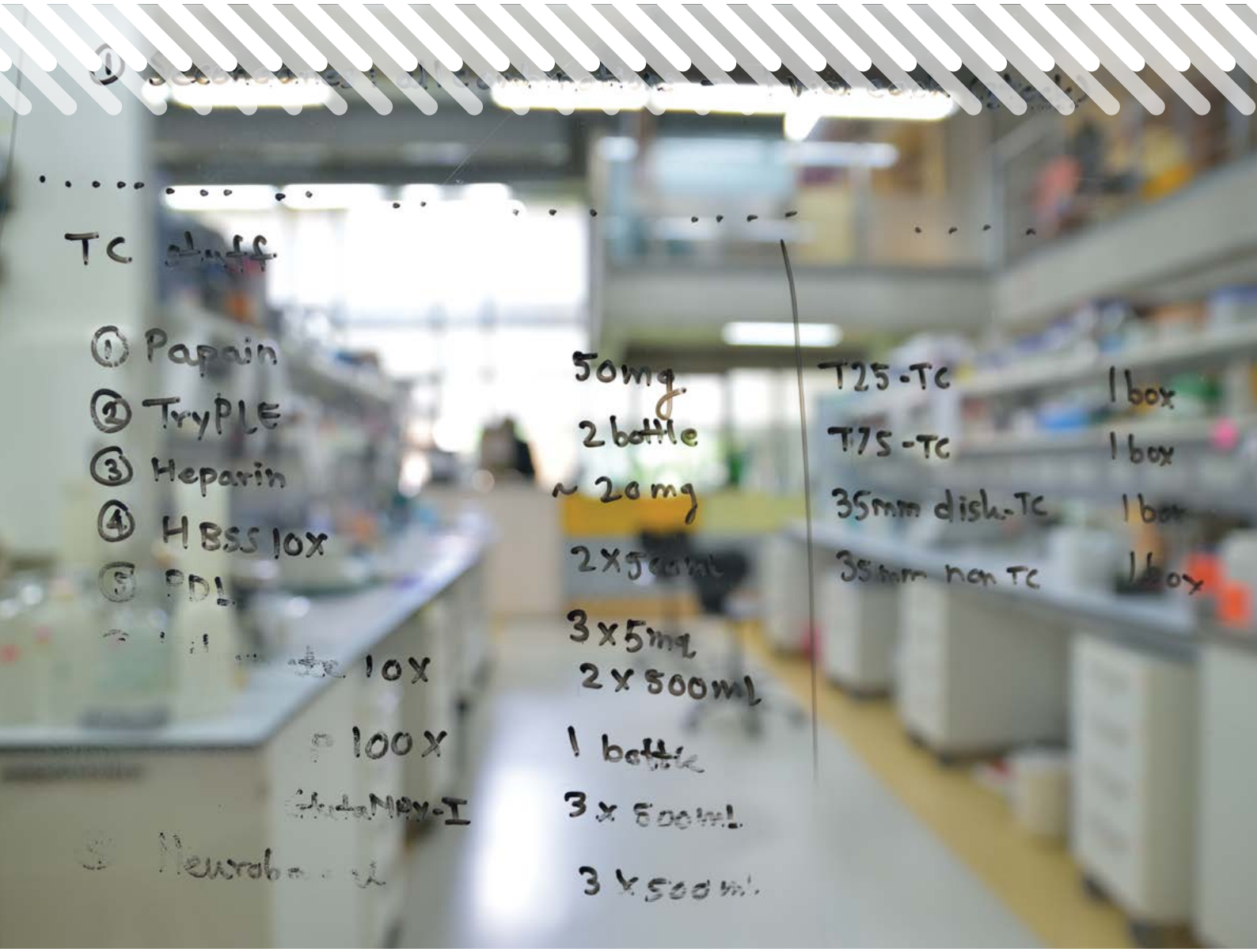
pertaining to Notch activity and interactions with cellular nutrient sensing machinery or sentinel metabolites, are under investigation in the laboratory.

Publications

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

Invited Talks

"Notch activity in T-cells and consequences to immune homeostasis" at the All India Cell Biology Conference at Thiruvananthapuram, India. 6 – 8th December, 2015



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7.2 *Arjun* Guha

REGULATION OF PROGENITOR CELL FATE DURING TISSUE MAINTENANCE AND INJURY-REPAIR

We are interested in the mechanisms that maintain progenitor cells in the adult animal and regulate their proliferation and fate in response to tissue damage

Epithelial tissues line the surfaces of organs throughout the body and serve to both nourish and protect. The maintenance and post-injury repair of these tissues is of vital importance. Some epithelia, such as the skin and the lining of the alimentary canal, have high rates of cell proliferation and turnover during homeostasis. In contrast, others such as the lining of the respiratory tract in the lung, have significantly lower rates but can dramatically upregulate proliferation in response to injury. We are broadly interested in the regulation of the proliferation and fate of progenitors that maintain and repair the epithelial lining of the respiratory tract. A long-standing narrative in lung injury-repair is that airway Neuroepithelial Bodies (NEBs) are a niche for specialised Club cells that are injury-resistant and can contribute airway repair in response to severe injury. The evidence in support of this narrative has been circumstantial since no specific markers have been reported for this cell population. Several years ago we showed that the NEB microenvironment in the fetal lung harbors a distinctive Club cell-like cell that can be distinguished by high levels of Uroplakin3a (Upk3a)-expression. Classical lineage analysis of Upk3a^{+} cells in the fetal lung revealed that they contribute to post-natal lung growth and generate both Club and ciliated cells to the adult lung (Figure 1, left panel). We have found that NEBs in the adult lung also harbor Upk3a^{+} Club cells (U-CCs). U-CCs in the adult lung are injury-resistant and contribute to airway post-injury repair. In addition, U-CCs also contribute toward airway maintenance long-term. Ongoing work focuses on understanding the distinctive features of these multipotent progenitors (U-CCs) and on the role of the NEB microenvironment as a progenitor niche.

Together with studies in the mouse model, we are also interested in the regulation of the progenitors of the adult respiratory system (tracheal system) of *Drosophila*. The transformation of a free-living larva to an adult fruit fly is dependent on several adult epithelial progenitor populations that remain quiescent until the onset of metamorphosis. We are currently utilising

photoactivateable cell-labelling methods to tag and follow the dynamics of adult tracheal progenitor cells during metamorphosis (Figure 1, right panel) and genetic approaches for understanding how these cells transition from a quiescent to a mitotically active state. In the long term, we will investigate whether these regulatory mechanisms are also utilised in the vertebrate lung.

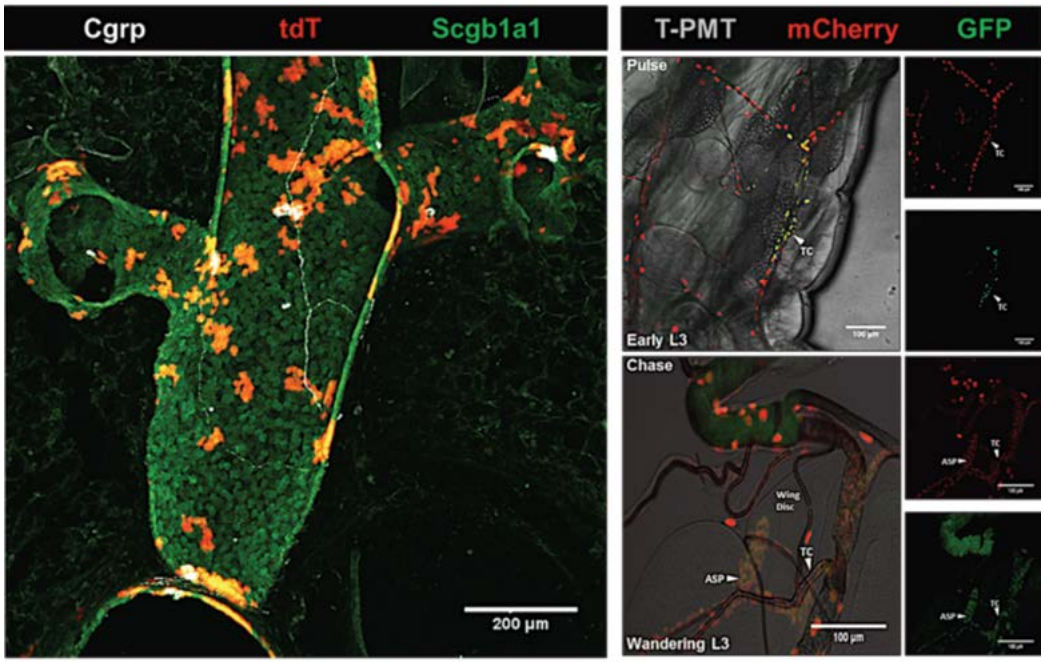

Fig 1

The left panel is 3D-reconstruction of a thick section of the mouse lung taken from an adult animal in which the Uroplakin3a-expressing cells were lineage-tagged in the embryo (E15.5). Progeny of the Uroplakin3a-expressing cells, marked by expression of Td-Tomato (red) are distributed throughout the airways and invariably associated with airway Neuroepithelial Bodies (marked by expression of Cgrp, white). The airways are marked by expression of Scgb1a1 (green). The right panel shows cells of the larval tracheal system in *Drosophila,* dual-labeled with a photoactivateable GFP and RFP (marked in yellow in the top panel), that proliferate and generate the primordia of the adult tracheal system (labelled ASP, bottom panel).

Publications

Rao, P.R., Lin, L., Huang, H., **Guha, A.,** Roy, S. and Kornberg, T.B. (2015). Developmental compartments in the larval trachea of Drosophila. ***eLife*** 2015;4:e08666

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7.3 *Sunil* Laxman

METABOLIC SENSING AND REGULATION OF CELL FATE

The metabolic state of a cell can directly control different cell fate decisions. Certain metabolites reflect metabolic state, and are sensed by "metabolic sensors". Our group identifies how specific metabolites (particularly amino acids), and their metabolic sensors regulate cell fate.

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

In prior work, we had discovered two conserved pathways that sense sulfur containing amino acids (methionine and cysteine, or SAAs), and S-adenosyl methionine, and regulate metabolism and cell growth. In one of these pathways, cells use a specific tRNA modification to sense SAAs. This specific tRNA modification is a thiolation modification on uridine (methoxycarbonylmethyl-thio uridine) that is present at the codon-recognition position. We found that tRNA thiolation was dynamic and depended upon intracellular SAA concentration. Thiolated tRNAs specifically increased translation capacity, while lack of tRNA thiolation increased SAA and lysine biosynthesis, thereby revealing the tRNA thiolation system as a regulator of SAA homeostasis, translation and growth. Several other recent studies also highlight the importance of SAAs in regulating cell fates, aging and survival. However, some very basic, fundamental questions remain,

including what makes methionine uniquely able to serve as a potent cue for growth, and how specific tRNA modifications can integrate metabolism, amino acid homeostasis and translation. Our current work addresses these questions directly. We predict that these mechanisms we identify in *S. cerevisiae* will remain highly conserved across eukaryotic cells, particularly in cells that show a high degree of plasticity. We also have expand our studies to investigate the metabolic basis of cellular co-operation, in complex but isogenic cell communities.

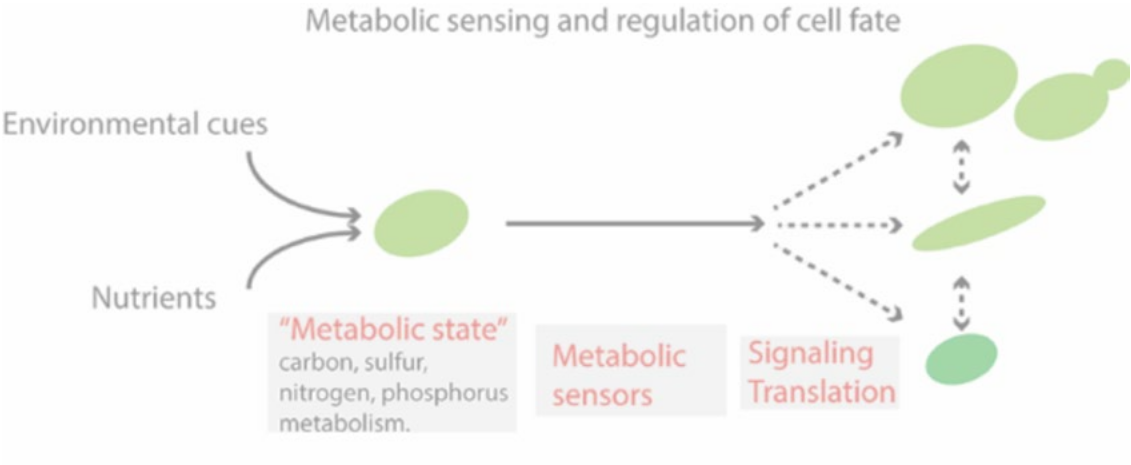

Fig 1

Metabolic sensing and regulation of cell fate

As part of the broader question of how amino acids and their sensors regulate cell fate, we are interested in the function of two major cellular organelles, the mitochondria and the vacuole. These organelles play central roles in regulating amino acid synthesis, storage and metabolic homeostasis, as well as provide the molecular building blocks that make up the cell. In this context, we have embarked on projects to identify how inter mitochondrial and vacuolar communication is regulated, and have identified mutants of specific proteins which result in a failure of appropriate inter-organellar communication, and are determining metabolic bottlenecks that these proteins regulate, as well as the signalling events they regulate. Part of this will be undertaken in a collaborative effort with the Frezza group at the MRC Cancer Unit at Cambridge, through a joint NCBS-inStem-Cambridge postdoctoral fellowship.

In a collaborative effort with the Kurzchalia lab at MPI-CBG, we have together identified the conserved role of a neglected but major metabolic pathway, the glyoxylate shunt, in enabling desiccation tolerance in the budding yeast S. cerevisiae and the nematode *C. elegans.* Our study also provides a central metabolic logic to explain the ability of some cells to survive without water (anhydrobiosis). We hope to extend this collaboration on future work in this regard.

To enable our broader studies of metabolic regulation, we have successfully developed novel, highly sensitive targeted approaches to quantitatively determine changes in metabolites (of specific, central metabolic pathways), as well as measure metabolic flux within these pathways.

Finally, we have embarked on an exciting collaborative effort within the RCF theme, to study the metabolic regulation of cell fate, focusing on T-cells. These studies are in collaboration with Dr. Apurva Sarin's group, where we have initiated studies to understand metabolic switches that regulate T-cell maturation and differentiation, and the roles of specific amino acids in T-cell maturation and function. Collectively, these studies hold tremendous promise in addressing a larger question on metabolic regulation of cell fates.

Publications

Erkut, C., Gade, V.R., **Laxman, S.** and Kurzchalia, T.V. (2016). The glyoxylate shunt is essential for desiccation tolerance in C. elegans and budding yeast. ***eLife*** 2016;10.7554/eLife.13614

Dhawan, J. and **Laxman, S.** (2015). Decoding the stem cell quiescence cycle - lessons from yeast for regenerative biology. ***J. Cell Sci.*** 128, 4467–4474

Invited Talks

"Waiting for go: post-transcriptional responses to specific amino acid limitation." ICYB 2015 conference, Kolkata, India. 13th December 2015

"Novel amino acid sensing and post-transcriptional response mechanisms"
"Bacterial Expressions" Simons-NCBS conference at Bengaluru, India. 3rd December 2015.

"Understanding cooperation in cell populations." Physics of Life, 3rd NCBS-Simons Monsoon School, Bengaluru, India. June 2015

"Amino acid sensors and metabolic switches that regulate cell fates." Max Plank Institute-CBG, Dresden, Germany. 22nd May 2015

"Amino acid sensors and metabolic switches that regulate cell fates." IFOM - FIRC Institute of Molecular Oncology, Milan, Italy. 15th May 2015

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7.4 *Tina* Mukherjee

SYSTEMIC AND METABOLIC CONTROL OF STEM CELLS

Our research is focused on identifying systemic and local metabolic cues controlling haematopoietic development, progenitor maintenance and function. Alongside identification, we are also interested in addressing the physiological relevance of these cues during development and stress conditions. Through these efforts we aim to highlight important signalling pathways, metabolic cues and their cross talk underlying haematopoietic cell fate decisions in homeostasis and upon alterations of environmental, metabolic, and stress conditions.

An important aspect of blood progenitors both in vertebrates (the common myeloid progenitors, CMPs) and invertebrates is accumulation of Reactive oxygen species (ROS) during development. This is important to prime the progenitors towards differentiation, but if uncontrolled, excess ROS can lead to oxidative stress and damage. How a system achieves a balance of ROS remains unclear. Our work in the *Drosophila* blood system reveals a metabolic mechanism by which cells can overcome this. We find that in this system, the progenitors utilise a variant TCA cycle, coupled to the GABA shunt. This programme in the progenitors enables a metabolic state that generates fewer energy rich intermediates and at the same time generates antioxidants that scavenge any excess ROS thereby enabling the ROS equilibrium to be achieved.

GABA-shunt driven metabolic pathways have been mainly described to function in bacteria, yeast and plants, where it enables cells with the capacity to combat oxidative stress and also kill pathogens. However, whether this holds true for higher organisms remains to be investigated. It will be interesting to see if tissue systems in vertebrates, such as haematopoietic stem cells or the insulin producing beta cells, which respond to GABA as a ligand and exist in oxidative

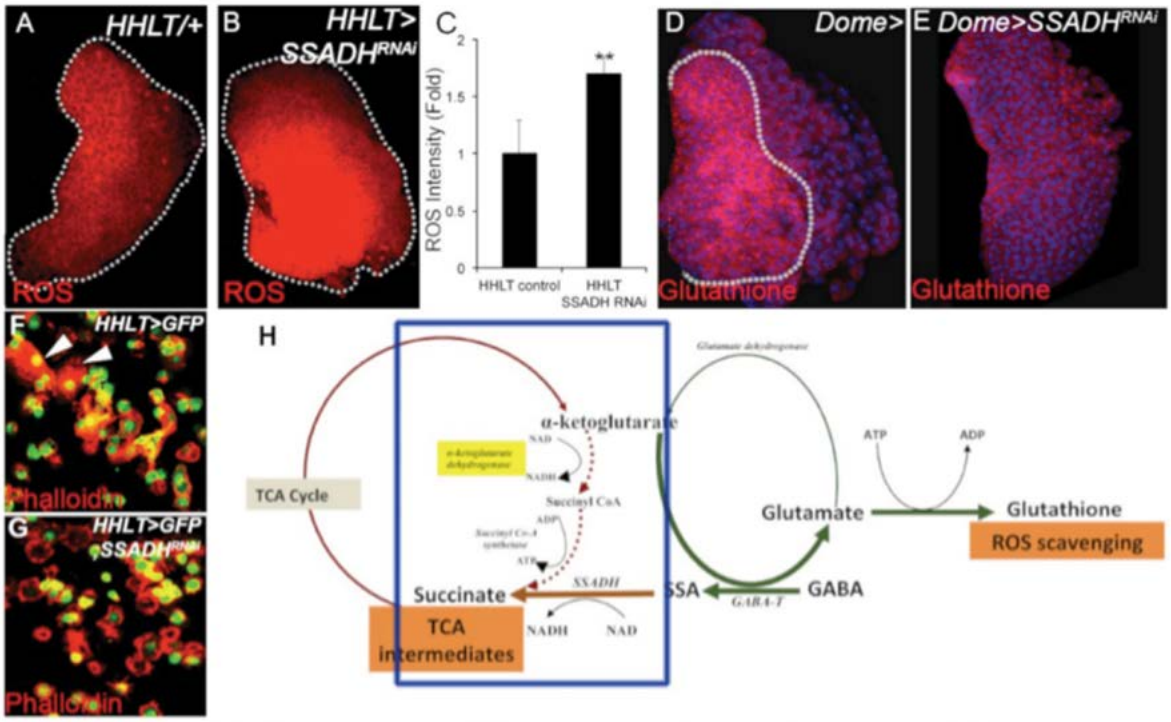


Fig 1

GABA metabolism maintains ROS and energy homeostasis in myeloid progenitors. Blood progenitors of the lymph gland lack key TCA-cycle genes (dashed arrows). GABA metabolism drives TCA and generates substrates for anitoxidant synthesis. Blocking GABA-shunt metabolic genes leads to excessive ROS accumulation (A-C) due to loss of Glutathione (red) synthesis in the progenitors (D and E). Additionally, GABA-shunt is also necessary to drive cellular responses during immune challenge. Lamellocytes (arrowheads) in F are missing in G. Proposed model (H).

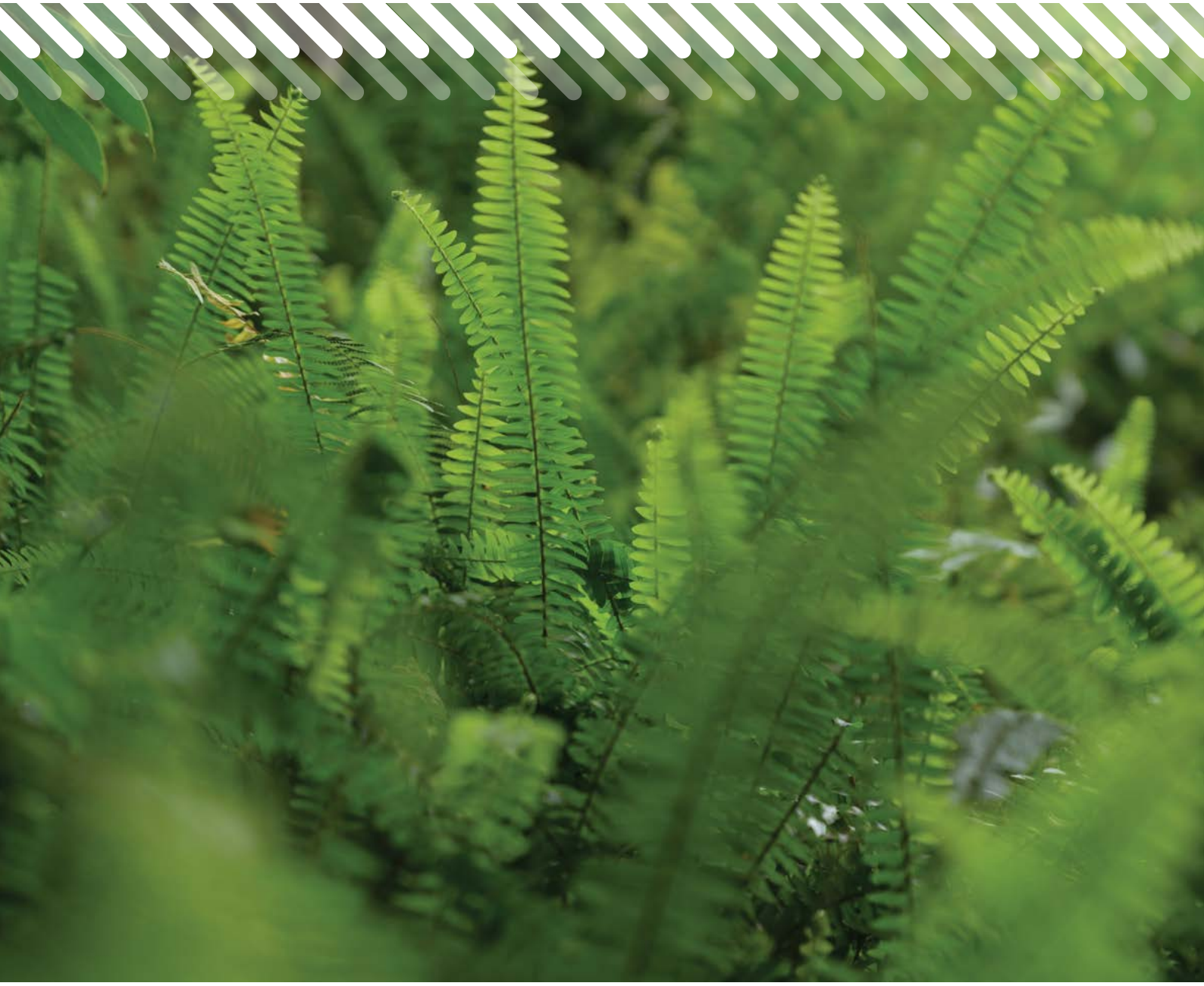
microenvironments can utilise GABA as a catabolite to combat the stressful microenvironment. This remains a question for future exploration. Taken together, our work highlights for the first time the importance of the GABA shunt in progenitor populations and that a "metabolic state" functions as a key paradigm in stem/progenitor cell maintenance, differentiation and function. In addition to investigating metabolic cues, our lab also employs genetic screening to identify novel systemic neuronal cues controlling blood development. In this context we have begun to identify new set of neurons that control blood progenitor development. Some of these cues function to maintain progenitors much like what we have identified in the past with GABAergic neurons. But the screen has also led to the identification of a group of neurons that signal to drive blood progenitor differentiation. These are interesting findings and highlight the importance of neuronal signalling and homeostasis within the nervous system as a key factor regulating haematopoiesis. We are currently exploring this in detail with the aim to identify the signal relayed from these neurons and their cross talk with GABA secreting neuronal cells. Our data is beginning to reveal important developmental controls of haematopoiesis via the neurons, both in maintenance and differentiation. As our future goal, we would like to address the physiology behind the different neuronal/systemic cues. We have interesting preliminary data to suggest that varying diets of *Drosophila* larvae or their exposure to different environmental stresses alters blood cell behaviour and physiology much like what we identify when neuronal signalling is perturbed. We would further like to investigate if neuronal signalling and the networks that we have identified, mediate extrinsic/environmental information to the blood system and regulate its development. This is an exciting possibility and future work in this direction will broaden our

view of blood stem/progenitor development, the cues they respond to, the inter-organ cross-talk between blood and the nervous system and the changes brought about in animal physiology.

Invited Talks

"Systemic control of stem and progenitor cell fate" at the InDRC at IIT-Kanpur, Kanpur, India. 23rd December 2015

"A Gal4 based lineage-tracing tool in Drosophila" at the InDRC, G-TRACE: at IIT-Kanpur, India. 21st December 2015



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7.5 *Jyotsna* Dhawan

QUIESCENCE AND ADULT STEM CELL POTENCY

The dhawan group is interested in the mechanisms by which the dormant or quiescent state of adult mammalian stem cells promotes the acquisition and maintenance of regenerative function. We use genome-wide strategies coupled with functional analyses to investigate the links between two key features of quiescence – repression of differentiation and the potential to return to active division.

While embryonic development is accompanied by high proliferative activity, in the adult, most cells have ceased cell division. Distinct arrested states exist: differentiated cells permanently withdraw from the cell cycle, but stem cells idle in a dormant state known as quiescence or G_0. These temporarily arrested progenitors are responsible both for maintenance of adult tissue, as well as regeneration after injury. De-regulation of quiescence underlies disease at opposite ends of a spectrum – cancer may represent a failure to enter arrest, while degenerative disease may represent a failure to exit arrest. Therefore, understanding the acquisition and maintenance of quiescence is experiencing renewed interest.

We use genome-wide strategies coupled with functional analyses to investigate the links between two key features of quiescence – repression of differentiation and the potential to return to active division. Using a culture system that models muscle stem cells, we have uncovered active controls at multiple levels of gene regulation specific to quiescence and use this framework to investigate a variety of adult stem cells. Our studies indicate that quiescent cells preserve two antagonistic programmes (division vs. differentiation) in an inactive but primed or poised state.

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

Chromatin and transcriptional mechanisms in G_0

• We have uncovered quiescence-specific mechanisms controlled by promoter-proximal pausing of RNA Pol II, and provide evidence that a large class of reversibly repressed genes are primed for transcriptional activation by stalled polymerase molecules. If genes that are normally stalled in G_0 but activated within minutes of reactivation are suppressed by RNAi, a failure of reactivation ensues, suggesting a key role for their poising in G_0.

• We are also studying the links between redox stress and muscle disease focusing on Selenoprotein N (SelN), in collaboration with Ana Ferreiro at CNRS. Selenoprotein N (SelN; encoded by SEPN1 gene) is associated to a human genetic disease, SEPN1-related myopathy (SEPN1-RM), typified by severe weakness and wasting of neck and trunk muscles. We have been investigating a role for SelN in regulating the cell cycle at distinct stages of myogenic differentiation: quiescent satellite cells, proliferating myoblasts, and differentiating and fusing myoblasts, where knockdown analysis provides evidence for stage-specific roles. A comprehensive transcriptome analysis suggests novel mechanisms in cell cycle control which we will investigate over the coming year.

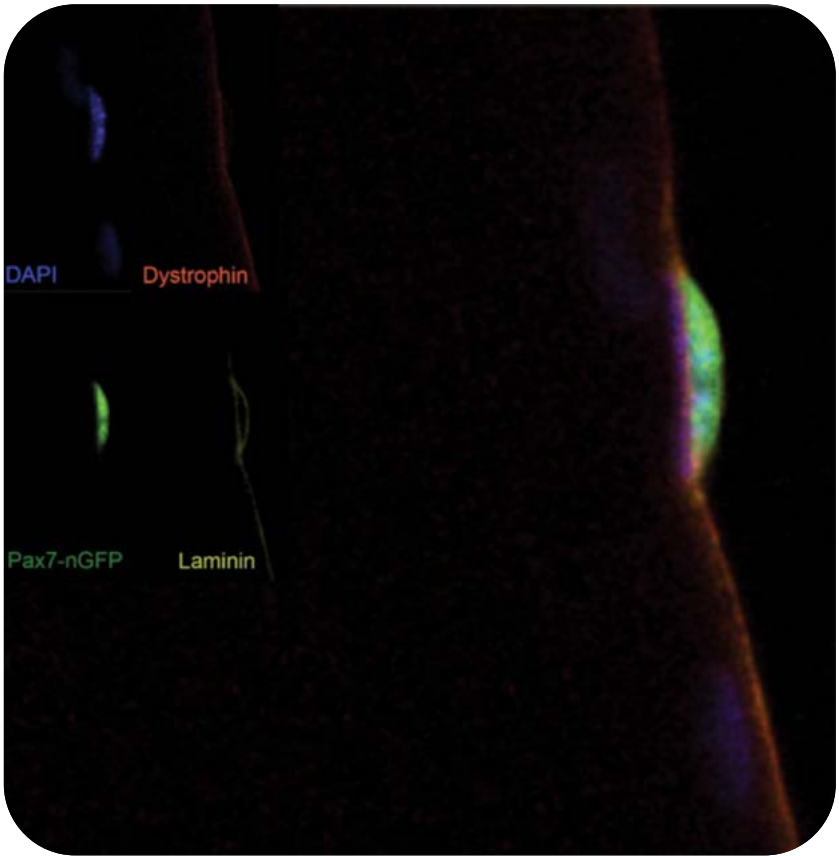

Fig 1

A satellite stem cell sits sandwiched between the basement membrane and the plasma membrane of the skeletal muscle myofiber.

Engineering quiescence by manipulating signals

Culture systems that permit human mesenchymal stem cells to be toggled between quiescence and activation *in vitro* have not been previously characterised. In collaboration with

Moustapha Kassem at Odense we have generated a new MSC quiescence model and uncovered transcriptional programmes associated with an altered secretory phenotype.

Using this model for generating quiescent human mesenchymal stem cells (hMSCs), we found that a receptor type protein tyrosine phosphatase (PTPR), a single pass trans-membrane protein, is induced during entry into G_0. Interestingly, this PTPR is known to regulate Wnt signalling via controlling a pathway that leads to phosphorylation of the transcriptional co-activator β-catenin. We report that this PTPR is involved in regulating balance between reversible and irreversible cell cycle arrest. Ablation of PTPR in adherent conditions suppresses proliferation and selectively induces the expression of pathway-specific differentiation regulatory genes. In quiescence, suppression of this phosphatase leads to a failure of reactivation upon adhesion, suggesting that it might be involved in preserving reversibility of cell cycle arrest in quiescent MSCs.

Publications

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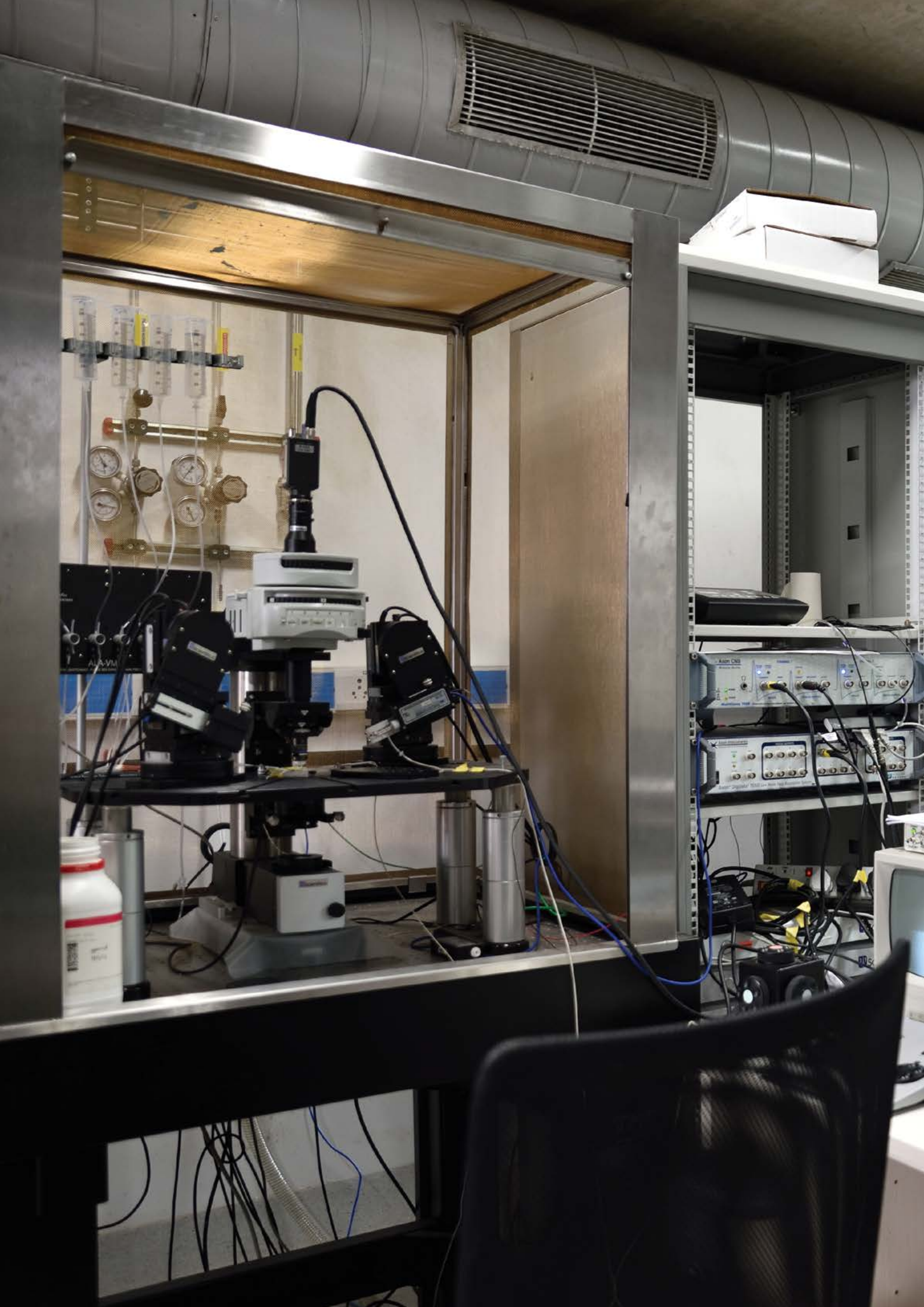

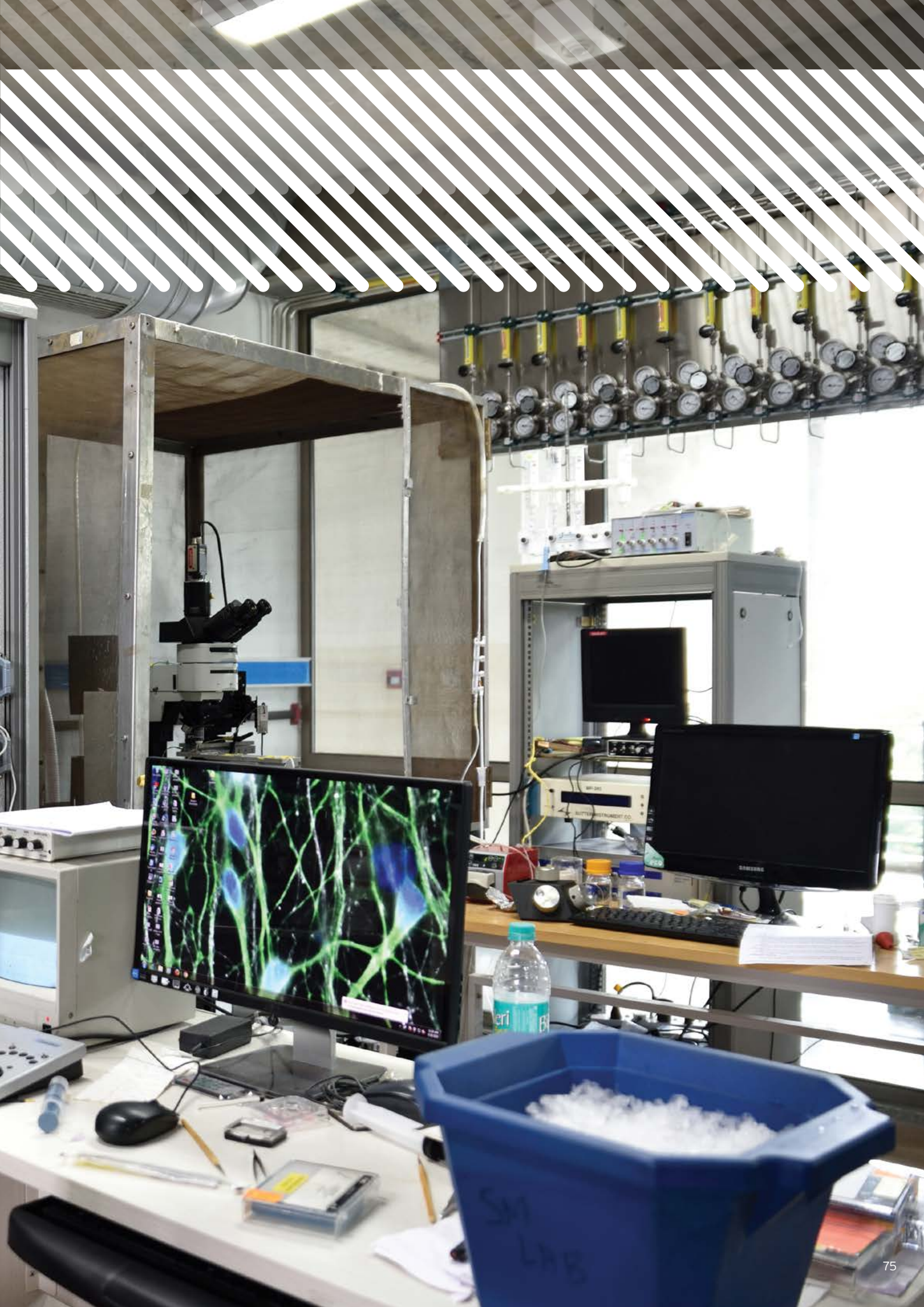

TA

8

Technologies for the Advancement of Science

Technologies for the Advancement of Science is a group with diverse strengths and research interests in disciplines ranging from vertebrate development biology to biophysics and chemistry of materials. The diversity of TAS is its strength, where investigators bring novel perspectives to challenging problems in biology. One of the core ideas behind the theme is to integrate programmes and catalyse new approaches and developments across biology. TAS has been successful in implementing this idea by generating many exciting intra-theme and inter-theme collaborations as well as interactions with other laboratories in the Bangalore Life Science Cluster (BLiSc).

While TAS is diverse, synergy generated within TAS has led to new projects and paradigms emerging through organic collaborations. The blend of material chemistry with regenerative biology has led to the development of an innovative transgenic technology for flatworms. This development would make flatworms, an excellent but unconventional model for regenerative biology, amenable to genetic manipulation and thus, enabling a new range of experimental approaches possible in this system. TAS has made advances in developing new self-assembled materials as well as fluorescent biosensors for visualising cell signalling. A collision of perspectives has led to an exciting discovery in light sensing and processing impacting how we think about eye evolution. Experiments here show that planarian flatworms, having a rudimentary, ancient eye, are able to discriminate between closely spaced colours. This finding challenges the conventional dogma that colour-specific photoreceptors are the basis of colour 'vision'. This and other findings have allowed groups in TAS to exploit natural light sensing as a functional readout to probe the trajectories / mechanisms underlying neural regeneration The value added by TAS and the outcome of the cross connections that have emerged is visible in the number of collaborative research projects with the TAS theme as the pivot; the progress of the research programmes of the individual groups is outlined in the following sections.

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8.1

S Ramaswamy
Jeff Abramson

MOLECULAR FORM AND FUNCTION LABORATORY

Our groups are interested in understanding the molecular basis of how biological molecules carry out their function. We do this by using a variety of biochemical and biophysical approaches. The group is primarily interested in the study of sugar metabolism and transport in bacterial and eukaryotic cells and to understand their role in health and disease. Often, structural studies are carried out to explain unique observations in nature, for example that of the adaptation of the fish walleye to increased uv radiation.

Structural studies of bacterial enzymes involved in sialic acid uptake and catabolism

Sialic acids (5-N-acetylneuraminic acids, Neu5Ac) are a family of related nine-carbon sugar acids that are used by bacteria for molecular mimicry, as nutrition, and in cell signalling. The motivation for this study is to understand how bacteria scavenge, transport and incorporate sialic acid into their lipooligosaccharides (LOS) or lipopolysaccharides (LPS). Bacteria incorporate this scavenged Neu5Ac into the LOS/LPS as the terminal non-reducing sugar. This sugar is recognised by the complement system in the serum as "self". Several opportunistic bacteria use this type of molecular mimicry to evade the immune system, resulting in serious pathological consequences. The enzymes from pathogenic bacteria that regulate the incorporation of sialic acid sugars onto the LOS/LPS are therefore important targets for therapeutic intervention. Our laboratory has earlier determined the structure of several periplasmic sialic acid binding proteins. In 2015-2016, we have crystallised three different enzymes (SiaB, NanK and NagB) that are involved in the metabolism of Neu5Ac in structure determination and structure function studies are now in progress.

Structural studies of membrane proteins involved in sugar and nucleotide-sugar transport

Na^+/glucose transporters (SGLTs) are integral membrane proteins, which co-transport Na^+ with sugars from the periplasmic space into the cytoplasm. According to the alternating access model for secondary active transporters, these proteins alternate between outward and inward-facing conformations during the transport cycle. The currently available structures—solved by our lab—from a bacterial homolog of SGLT are in the substrate-bound inward- occluded and the substrate-free inward-open conformations. Utilising new techniques like conformational specific crystallisation chaperones that specifically bind to alternative conformations we hope to develop a complete model for alternating access. In addition the human transporters are therapeutic targets for treating type II diabetes and we have isolated pure human protein.

A large majority of membrane and secreted proteins in eukaryotes are glycosylated. These proteins are glycosylated in the endoplasmic reticulum (ER) and the Golgi apparatus. Nucleotide sugar transporters of the SLC35 (Solute Linked Carrier) family transfer activated sugars into the ER and the Golgi apparatus. These genes are found in all eukaryotes and previous studies of multicellular organisms deficient in nucleotide sugar transporters have shown these transporters to be involved in development and cancers. In spite of considerable genetic and biochemical data available on these proteins, there is no three dimensional structure of this family of proteins. Our goal is to use an orthologue approach to express, purify, crystallise and carry out structure-function studies of nucleotide sugar transporters from various species. This approach has led to the expression, purification and production of crystals that diffract to 7.0 Å resolution.

Molecular explanations for observations in nature

We pursued a project to find a molecular explanation for the interesting observation that some yellow walleye fish turn blue in colour when exposed to UV radiation. Our studies on the protein that causes the blue colour show how this protein absorbs in the UV region (the damage causing radiation) and emits as fluorescence in the red region (that does not cause damage).

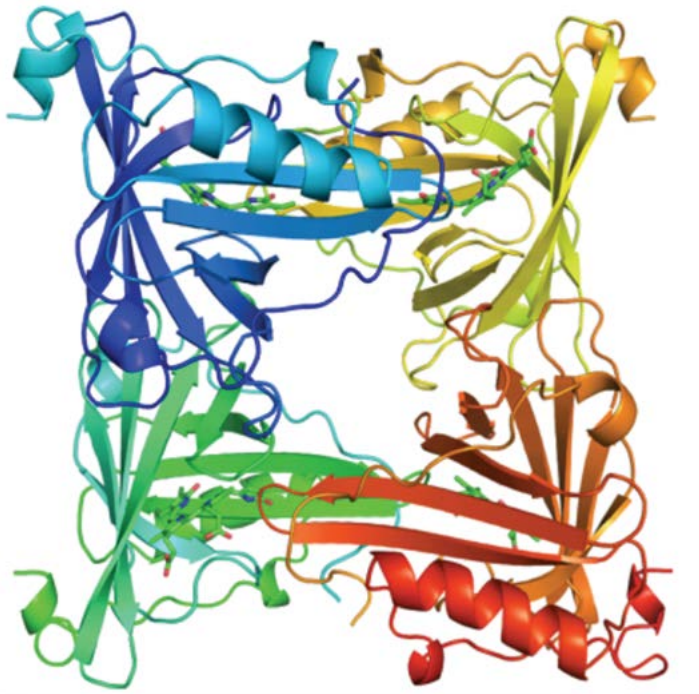

Fig 1

The tetrameric structure of the wild type sandercyanin fluorescent protein determined by X-ray crystallography. The protein structure is represented as a cartoon and the ligand biliverdin is represented as ball-and-sticks model.

In another project, an observation that the viviparous cockroach, *Diploptera punctata* provides food to its embryos in a crystalline form, led us to isolate these crystals and determine their structure. The first crystal structure of the *ex-cellulo,* naturally grown *in vivo* crystals reveals a lipid binding protein that is heterogenous in sequence and highly glycosylated.

Publications

Banerjee, S., Coussens, N.P., Gallat, F.-X., Sathyanarayanan, N., Srikanth, J., Yagi, K.J., Gray, J.S.S., Tobe, S.S., Stay, B., Chavas, L.M.G. and **Ramaswamy, S.** (2016). Structure of a heterogeneous, glycosylated, lipid-bound, *in vivo*-grown protein crystal at atomic resolution from the viviparous cockroach Diploptera punctata. ***IUCrJ*** 3, 282–293

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8.2 *Akash* Gulyani

PROBING WITH LIGHT: BIOSENSORS FOR CELL SIGNALLING AND NATURAL LIGHT SENSING FOR NEURAL REGENERATION

Our lab has developed new ways to visualise and understand dynamic processes in single cells as well as whole organisms. We study cell signalling and neural patterning using 'innate' light sensing, fluorescent probes, bioengineering and chemical biology. Our vision is to integrate knowledge from multiple disciplines to address complex problems in cellular and regenerative biology.

Visualising signalling dynamics in living cells

Cell signalling is complex, with critical signalling proteins controlling multiple cellular processes and cellular outputs. It is not clear how such precision is achieved. Further, the activity of key signalling proteins is highly regulated – proteins may be activated only in specific intracellular locations and for defined time periods. To address spatial and temporal dynamics of protein activity, we develop fluorescence-based biosensors that visualise such activity in live cells and tissues. One area of focus is signalling regulated by Src family kinases, non-receptor tyrosine kinases that are critical in mediating cell migration, proliferation and fate, including stemness and differentiation. Src kinases are directly involved in major diseases including cancer, inflammation and heart diseases. While Src kinases are much studied, it is not clear as to how these kinases control multiple signalling pathways, often simultaneously. Also, the function of individual kinases within the family has been hard to decipher, with a fine balance between specific and redundant roles.

We have generated fluorescent biosensors that can specifically report on the activity of individual Src kinases in living cells. For this, we have established a new platform for making fluorescent biosensors at inStem. In our method, using high-throughput screening and protein chemistry, we engineer protein binders (monobodies or antibody mimics) that can bind and sense active kinase in living cells. These monobodies are conjugated with specific dyes and fluorophores to generate sensitive sensors that respond to target binding. Using our new approach, we have now generated sensitive sensors for the critical Src kinase, Fyn (Figure 1). These sensors are built on the principle

of fluorescence resonance energy transfer (FRET) where the binding of the sensor to the kinase results in increased FRET signal. We have validated our new sensors in cellular experiments and have shown that our sensors can report on spatially localised Fyn kinase activity in living cells. This is the first imaging/visualisation of Fyn activity in live cells. With our collaborator, Dr. Colin Jamora, we have also shown that Fyn kinase is critical for cell migration in a model for fibrosis. Fyn kinase is critical in cancer, cell migration, cell fate, mechanical signalling as well as in neural activity. Using our new sensor, we have demonstrated how Fyn activity is tightly regulated and peaks at the cell edge. We are now studying the pattern of Fyn activity in response to distinct stimuli. Since our approach is very general, we are also generating fluorescent biosensors for c-Src and c-Yes. Our work will be aimed at gaining a comprehensive understanding of how critical Src family kinases regulate cellular outputs through spatio-temporally regulated activity in distinct intracellular compartments.

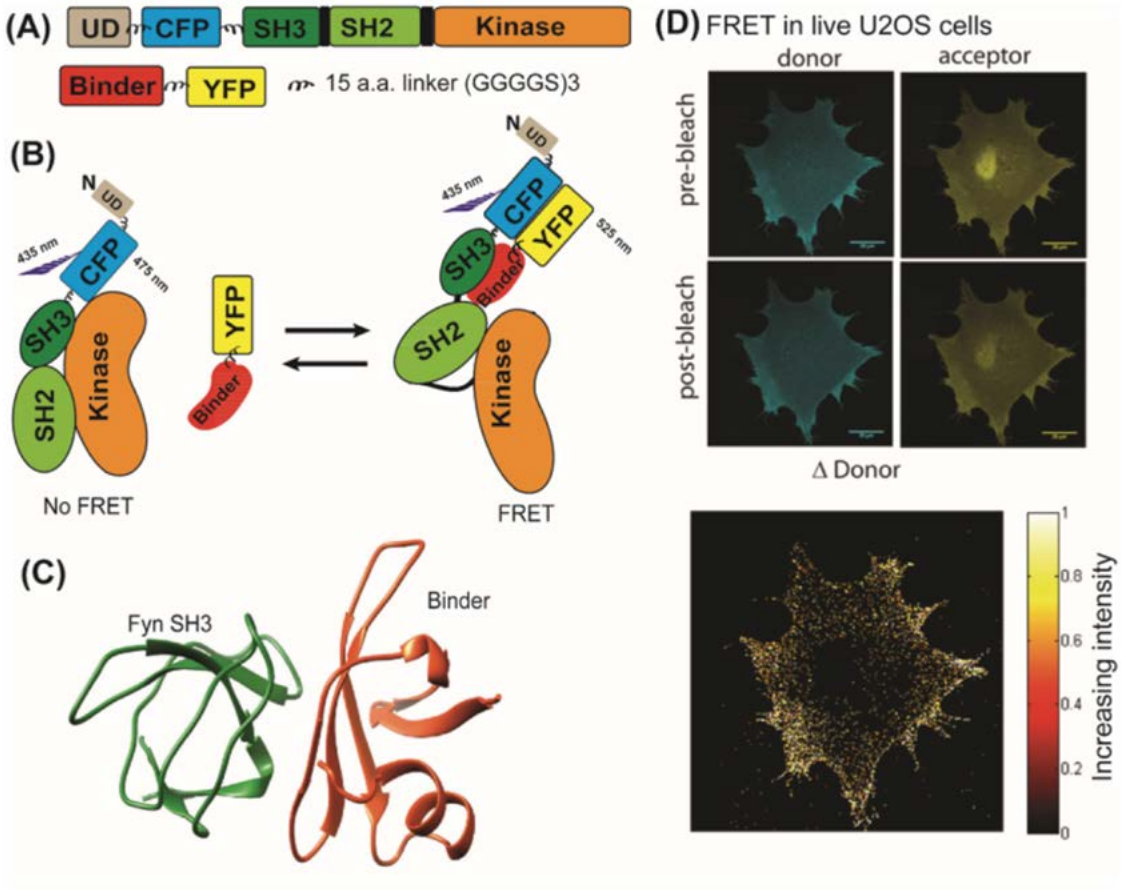

Fig 1

Imaging Src family kinase, Fyn activation dynamics in living cells. Novel engineered fluorescence resonance energy transfer (FRET) biosensors allow visualisation of Fyn activity in live cells. A) Biosensor is based on a binder (in red) for active Fyn. Domain organisation of Fyn kinase with design for FRET based sensor. Cyan fluorescent protein (CFP) is inserted in Fyn while binder for active Fyn is labelled with yellow fluorescent protein, YFP. B) Concept and design principle governing Fyn activity sensor. In the inactive state Fyn shows a closed conformation, with intramolecular interactions (left). This closed form does not bind the labelled binder, leading to a situation with no FRET. When active, kinase is more 'open' with the SH3 domain more accessible. The binder is able to bind to this active, open form and show FRET when the kinase is active in cells. C) Ribbon diagram showing NMR solution data of Fyn SH3 complex with binder. F29. D) Data showing FRET based readout for Fyn activity using the new biosensors. This data was obtained by recording FRET based on the methodology of fluorescent acceptor photobleaching.

Shedding 'light' on neural regeneration

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

Planarians possess one of the first examples of an eye and a 'brain' in evolution, and are highly light sensitive. We focused on examining planarian light sensing in considerable detail and have now been able link novel aspects of light sensing to regeneration and functional recovery. We could show that contrary to previous knowledge, planarians can resolve between very closely related light inputs with only a rudimentary light sensing system. Our work shows that the planarian cerebral eye and nervous system has the ability to accomplish 'comparative processing'. Interestingly, during regeneration of the eye and the brain, we can separate this ability of the neural network to 'process' or compute from simple light sensing. This opens a new area of research where we can address the molecular and structural mechanisms underlying neural regeneration and function with our collaborator, Dr. Dasaradhi Palakodeti. Our assays also allow us to ask, what is the minimal requirement to carry out comparative processing in a regenerating nervous system? Towards this end, we have carried out detailed imaging of the planarian brain and the eye in regeneration. Light sensing assays and imaging allows us to map eye and neural regeneration in new ways.

Invited Talks

"Hierarchical light sensing and processing revealed through planarian regeneration.", Indian Society for Developmental Biology Biannual meeting, Institute for Genomics and Integrative Biology, IGIB, Delhi, India. July 2016.

"'A sense of light?' Visualizing cellular dynamics and neural regeneration through optical methods", InStem Annual review of research, Bengaluru, India. March 2016.

"Probing cellular signaling and regeneration", Regional Centre for Biotechnology, RCB, Delhi, India. 16th February 2016.
"'A sense of light?' Visualizing neural regeneration and cellular dynamics", Institute for Genomics and Integrative Biology, IGIB, Delhi, India. 15th February 2016.

"Illuminating the landscape of neural regeneration: Hierarchies in innate light sensing and processing", International Meeting on Flatworm Biology, Oxford, UK. August 2015.

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8.3 *Praveen* Vemula

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

The Vemula group develops a wide range of self-assembled biomaterials to solve unmet clinical needs. At present, his lab is focused on developing inflammation-responsive and inflammation-targeting hydrogels as well as microparticles for localised delivery of drugs to ameliorate colon inflammatory disorders.

Efficient treatment of fluctuated inflammatory and autoimmune diseases is majorly limited by the ability to deliver drugs in a safe, responsive and targeted manner. The lack of targeting ability to inflamed tissue is a hallmark to deliver drugs for efficient treatment of localised inflammatory disorders such as inflammatory bowel disease (IBD).

Inflammation-targeting hydrogel microparticles for the treatment of gut inflammatory diseases: Inflammatory bowel disease (IBD) in its two main variants, ulcerative colitis (UC) and Crohn's disease affects several million people around the world. One approach to developing safer and more efficient therapies would be inflammation-targeted drug delivery to deliver high drug concentrations locally at the site of inflammation with minimal exposure to healthy tissue. Enemas as a basic form of targeted drug delivery to the inflamed colon are routinely used in mild-to-moderate ulcerative colitis. However, typical enema-based formulations require the patient to retain the enema for long periods of time, which is difficult when suffering from diarrhoea. The need for frequent dosing negatively affects patient compliance. Additionally, high concentrations of active drug may result in systemic side effects.

Our strategy to target inflammation is to develop drug delivery systems that exploit specific features of the diseased tissue. Inflammation of the colonic mucosa leads to the depletion of the mucus layer and *in situ* accumulation of positively charged proteins including transferrin, bactericidal protein, and anti-microbial peptides. This leads to the build-up of positive charges at the damaged epithelial surface, providing a target and anchor for drug carriers with negative

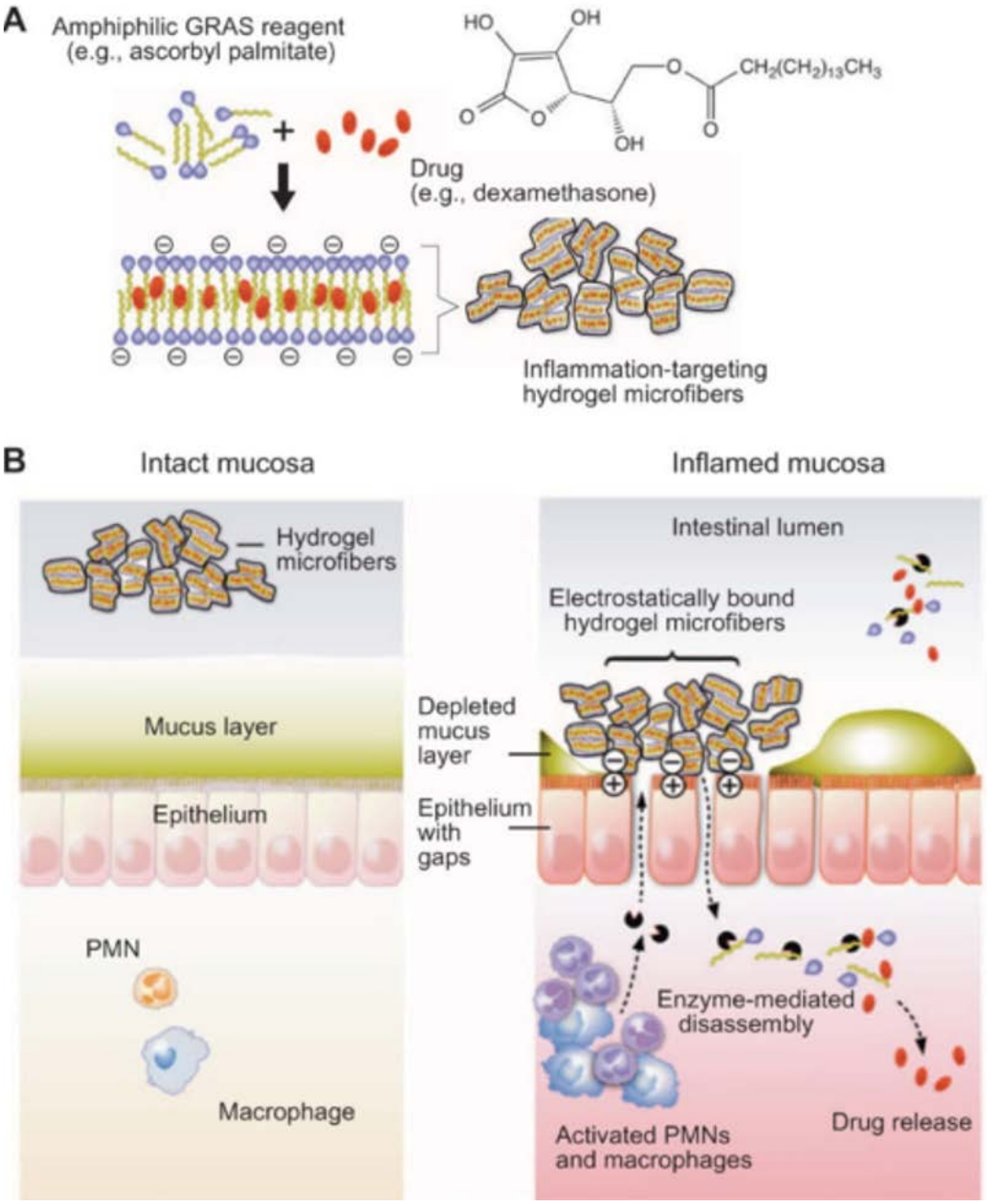

Fig 1

A) Schematic for self-assembly of Asc-Pal amphiphile to form hydrogels and encapsulation of drugs.
B) Inflammation leads to the depletion of mucus layer and accumulation positive charge at site of inflamed tissue. Hydrogel microfibers that have a negatively charged surface can preferentially adhere at inflamed tissue and release drugs in response to proteolytic enzymes.

surface charge. Additionally, inflammation is accompanied by up-regulation and secretion of proteolytic enzymes including matrix-metalloproteinases (MMPs) and esterases. A drug delivery system with an overall negative surface charge and containing an enzyme-labile linker should therefore preferably adhere to the inflamed mucosa and release drugs in response to proteolytic

enzymes at the site of inflammation. Furthermore, binding of the drug delivery system to the mucosa should prolong local drug availability and permit a reduction in dosing frequency.
We have developed an amphiphile, ascorbyl palmitate (Asc-Pal), which is generally recognised as a safe chemical agent by the US Food and Drug Administration. We have optimised conditions to generate a hydrogel through self-assembly of Asc-Pal. Asc-Pal also has an esterase enzyme-labile bond that could be cleaved in an inflammatory environment (Figure 1).

We have developed a hydrogel made from Asc-Pal that can be used as an inflammation-targeting hydrogel (IT-hydrogel) for drug delivery in IBD (Figure 1). IT-hydrogel microfibers encapsulate hydrophobic drugs and, owing to their negative surface charge, preferentially adhere to the inflamed mucosa in two murine colitis models, T-bet$^{-/-}$Rag2$^{-/-}$ ulcerative colitis (TRUC) and dextran sulfate sodium (DSS)–induced colitis, as well as to tissue samples from patients with UC. Using the corticosteroid dexamethasone (Dex) as a model drug, we demonstrate that drug-loaded IT-hydrogel microfibers administered to colitic mice via enema are therapeutically more efficacious and result in less systemic drug exposure than free Dex. Our study provides proof of concept for IT-hydrogel as a safe and potentially effective drug delivery platform for colonic IBD and other inflammatory diseases.

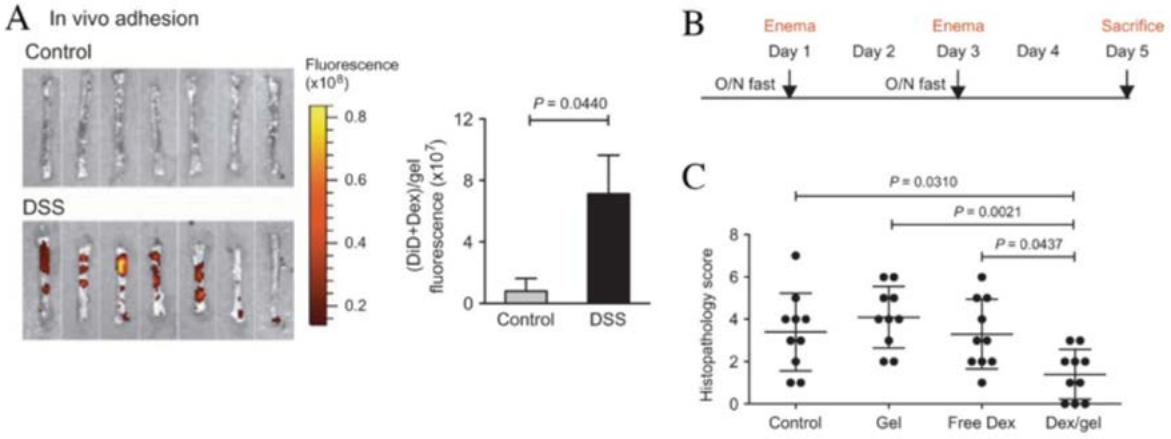

Fig 2

A) Fluorescence images of colons. WT mice with DSS-induced colitis and healthy controls received an enema of (dye+Dex)/gel. The animals were sacrificed after 12 hr of gel administered, and fluorescence of the distal colon was measured (left). B) Design of animal experiment. C) Histopathology scores after treatment with enemas of IT-hydrogel (Gel), free Dex, or Dex-loaded IT-hydrogel (Dex/gel, 70 mg of Dex equivalent per dose in both groups). Control mice received no treatment. Data are means ± SD (n = 10 mice per group). P = 0.0029 by one-way ANOVA; comparison of individual groups by Tukey post hoc test.

Inflammation-targeting hydrogel preferentially adheres to inflamed mucosa in mice with colitis: We have examined the adhesion of IT-hydrogel to inflamed colon epithelium using two established mouse IBD models: chemically induced DSS colitis and the spontaneous TRUC model. Wild-type mice with DSS colitis and untreated controls, or TRUC and Rag2-/- control mice received a single enema of dye (DiD) encapsulated gel. Animals were sacrificed 12 hrs later, the distal colon was removed, and fluorescence retention was quantified. Colons from colitic mice demonstrated more gel adherence compared to the respective controls in both models (Figure 2A). Administration of free dye via enema to mice with DSS colitis did not result in retention of the fluorescence signal when the colon was analysed 12 hr later. Thus, we have demonstrated that IT-hydrogel microfibers preferentially adhere to the inflamed colon mucosa mediated by electrostatic interactions.
Drug delivery via IT-hydrogel enema improves therapeutic efficacy: Colitic TRUC mice received

an enema of Dex-Pal-loaded IT-hydrogel (Dex/gel) or water-soluble Dex-21 phosphate in PBS (free Dex) on experimental days 1 and 3. Untreated mice (Control) and mice that received gel without drug (Gel) served as controls. All mice were sacrificed on day 5 for blinded histopathological analysis of the colon by a board-certified gastrointestinal pathologist. Disease severity was significantly reduced in mice given Dex/gel (mean colitis score, 1.4) compared to all other experimental groups, whereas mice in the free Dex group (mean colitis score, 3.3) did not differ significantly from untreated mice (mean colitis score, 3.4) or mice that had received gel only (mean colitis score, 4.1) (Figures 2B and 2C).

In conclusion, we have developed a strategy for targeted drug delivery to the inflamed colonic mucosa using hydrogel microfibers prepared from an amphiphilic GRAS reagent. Through attaching to the inflamed mucosa and selectively releasing drug at the site of inflammation, this system has the potential to prolong local drug availability, minimise systemic drug absorption, reduce dosing frequency, and lower the burden on the patient for retaining enemas after administration, all of which should improve compliance, reduce the risk for systemic toxicity, and maximize therapeutic efficacy.

Publications

Saha, P., Yeoh, B.S., Singh, R., Chandrasekar, B., **Vemula, P.K.,** Haribabu, B., Vijay-Kumar, M. and Jala, V.R. (2016). Gut Microbiota Conversion of Dietary Ellagic Acid into Bioactive Phytoceutical Urolithin A Inhibits Heme Peroxidases. ***PLoS One*** 11, e0156811

Amit, I., Baker, D., Barker, R., Berger, B., Bertozzi, C., Bhatia, S., Biffi, A., Demichelis, F., Doudna, J., Dowdy, S.F., et al. (including Vemula, P.K.) (2016). Voices of biotech. ***Nat. Biotechnol.*** 34, 270–275

Zhang, S., Ermann, J., Succi, M.D., Zhou, A., Hamilton, M.J., Cao, B., Korzenik, J.R., Glickman, J.N., **Vemula, P.K.,** Glimcher, L.H., et al. (2015). An inflammation-targeting hydrogel for local drug delivery in inflammatory bowel disease. ***Sci. Transl. Med. 7,*** 300ra128–ra300ra128

Lalitha, K., Muthusamy, K., Prasad, Y.S., **Vemula, P.K.** and Nagarajan, S. (2015). Recent developments in β-C-glycosides: synthesis and applications. ***Carbohydr. Res.*** 402, 158–171

Sen, D., **Vemula, P.K.,** Jayandharan, G.R. (2015). Intra-articular gene transfer of miR-15b attenuates molecular mediators of haemophilic arthropathy in a murine model of haemophilia. ***Mol. Therapy 23,*** S96-S97

Invited Talks

"Smart materials: Inflammation-responsive smart biomaterials to protect transplanted organs" at the Indo-Germany Frontiers of Engineering symposia at Postdam, Germany. 19 – 22nd May 2016

"Inflammation-responsive biomaterials to protect transplanted organs" at the BiTERM-2016 Conference on Biomaterials, Biodiagnostics, Tissue Engineering, Drug Delivery and Regenerative Medicine at IIT-Delhi, Delhi, India. 15 – 17th April 2016

"Nanomedicine: A new paradigm in drug delivery – Disease-responsive and targeting drug delivery in healthcare" at the Bengaluru NANO INDIA, at Bengaluru, India. 5th March 2016

"Disease-responsive biomaterials to prevent autoimmune diseases" at the TIFR-Centre for Interdisciplinary Sciences at Hyderabad, India. 20th August 2015

"My journey from mentee to mentor – A discussion on career plan" at the Indian Institute of Technology, Kanpur, India. 21st July 2015

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8.4 *Dasaradhi* Palakodeti

UNDERSTANDING POST-TRANSCRIPTIONAL REGULATION IN STEMNESS AND DIFFERENTIATION

The primary focus of our lab is to study post-transcriptional regulation of stem cell function and tissue organisation in metazoans. We have demonstrated that translational regulators such as microrna and trna derived small rnas (tsRNA) regulate tissue organisation and stem cell fate decisions in metazoans.

Planar cell polarity and axon guidance cues guiding cephalic ganglia and photoreceptor regeneration in planarians are regulated by *miR-124c*

The unique capacity of planarians to regenerate their central nervous system provides an opportunity to investigate neural differentiation, neural patterning, organisation and scaling of the brain and photoreceptors. Multiple studies have identified microRNAs as key regulators of diverse cellular processes in metazoans. Among the several miRNAs discovered, the *miR-124* family of microRNAs is highly conserved and is known to regulate neurogenesis by facilitating neural differentiation. Here, we studied the role of *miR-124c* in planarian brain regeneration and neural patterning. We have shown that *miR-124c* is expressed on day 3 post-amputation in the anterior regenerating tissue of planarians. It regulates key pathways such as axon guidance and planar cell polarity essential for accurate neural patterning and neural growth during anterior regeneration. We also showed that *miR-124c* regulates *slit-1,* an axon guidance protein expressed in the midline, by controlling the Notch pathway (in collaboration with Prof. Alejandro Alvarado Sanchez). Together, our results reveal a novel role for *miR-124c* in regulating axon guidance cues and the planar cell polarity pathway. This is essential for precise neural growth and neural wiring, which is necessary for the regeneration of both, a functional brain, and photoreceptors.

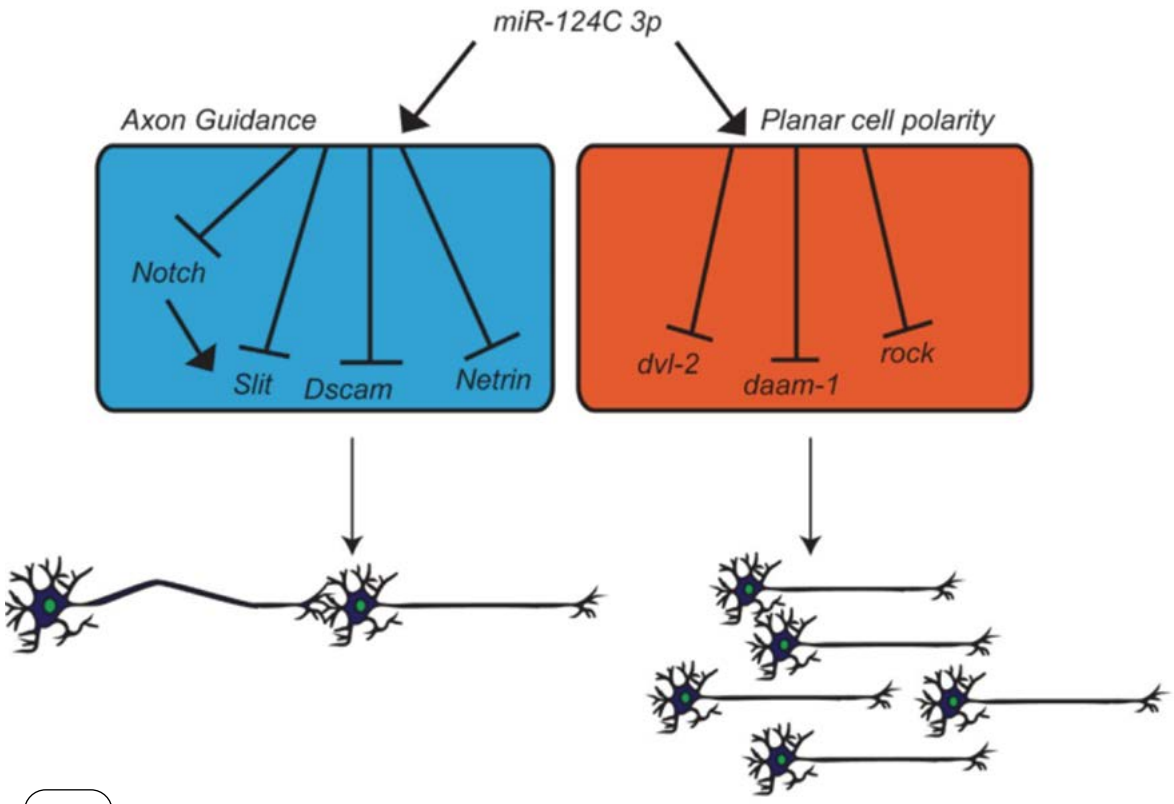

Fig 1

Schematic showing the genes directly regulated by miR-124c during planarian regeneration. Mir-124c regulate genes encoding for axon guidance proteins and planar polarity pathway essential for neural patterning and brain organisation in planarians.

Stem cell fate switch is regulated by tRNA derived small RNAs.

Cellular differentiation is a complex process. Transitions from a stem to differentiated state require control of gene expression at transcriptional, post-transcriptional and translation level. Studying the regulatory mechanisms in this process will help understand the biology of cellular transitions. This work explores the control of cellular transitions at the post-transcriptional/ translational level by small RNAs using stem cell differentiation as a paragon for cell state transitions.

In collaboration with Dr. Srikala Raghavan from inStem and Dr. Ramanuj Dasgupta from A*Star, Singapore, we identified small RNAs that were enriched upon differentiation by comparing the small RNA profiles from mouse embryonic stem cells (ESCs) and retinoic acid (RA) induced differentiating ESCs. Upon further probing, these small RNAs of ~33nt mapped to tRNAs, specifically to the 5′-tRNA half. Interestingly, these small RNAs seem to be produced in abundance during early differentiation and reached levels comparable to the stem cell after complete differentiation. High-throughput sequencing of isolated fractions from polysome profiles revealed associations of tsRNAs in translating pools (80S and Polysome) suggesting a strong role for these tsRNAs in fine-tuning translation. Remarkably, *in vitro* experiments using tsRNA mimics confirmed tsRNAs as translation repressors. Electron microscopy of the in vitro translation lysates treated with tsRNA mimics showed formation of large aggregates suggesting that they could form large translational repression complexes. This data is supported by the protein interactome identified through tsRNA pulldowns. *In vitro* mRNA pulldowns using tsRNA mimics reveal associations of tsRNAs with pro-stemness transcripts during differentiation and vice versa.

Similar tsRNA enrichments were observed in other systems such as epidermal stem versus non-stem compartments and cancer lines suggesting generic roles for these tsRNAs in translational regulation. Together, we propose a role for tsRNAs in translational control/stalling of specific pools of mRNAs to facilitate efficient cellular differentiation.

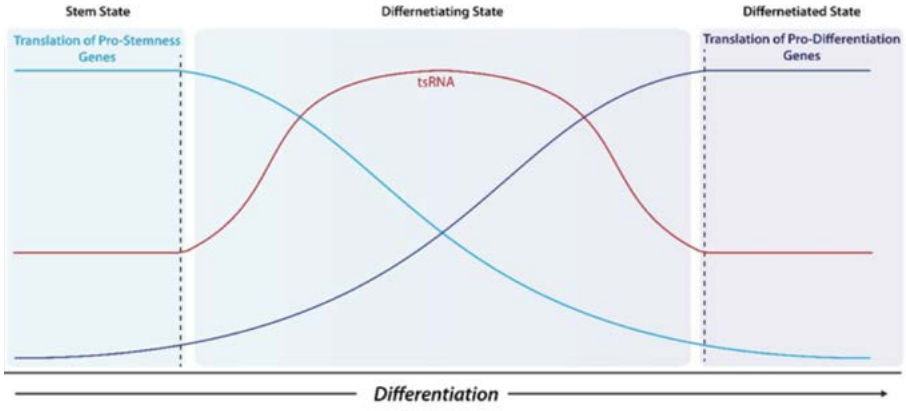

Fig 2

Pictorial representation of tRNA derived small RNAs function in stem cell fate switch.

Publications

Natarajan, N., Ramakrishnan, P., Lakshmanan, V., **Palakodeti, D.** and Rangiah, K. (2015). A quantitative metabolomics peek into planarian regeneration. ***Analyst.*** May 21;140(10):3445-64

Rao, R.A., Dhele, N., Cheemadan, S., Ketkar, A., Jayandharan, G.R., **Palakodeti, D.** and Rampalli, S. (2015). Ezh2 mediated H3K27me3 activity facilitates somatic transition during human pluripotent reprogramming. ***Sci. Rep.*** 5, 8229

Invited Talks

"MicroRNAs and Brain regeneration in planaria" at RNA meeting held at CCMB, Jan 8th- 10th, 2016.

"Sme-miR-124c is essential for brain regeneration and neural patterning in planarians." at International flatworm meeting held at Oxford University from Aug 3rd till 6th, 2015.

"Multicellularity, Stem Cells and Regeneration", Guest lecture to school students on science day held at JNCASR, Bengaluru in 2015.

"MicroRNA, miR-124 is essential for cephalic ganglion patterning and organization of optic chaism during planarian regeneration." at InSDB meeting held at CCMB, Hyderabad, July 15th -18th, 2015.

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8.5 *Ramkumar* Sambasivan

GENETIC REGULATORY MECHANISMS UNDERLYING VERTEBRATE DEVELOPMENT AND EVOLUTION

During development, a network of signalling pathways and transcriptional factors control the functional specialisation of embryonic cell types into various tissues and organs. We study such gene networks to gain mechanistic insight into vertebrate development and evolution.

Generating the animal body plan during embryonic development requires positional information in order to achieve the typical spatial pattern of organs and tissues. In bilaterians (animals with bilateral body symmetry), the regulatory programme that governs patterning of the body axes, anterior-posterior (A/P) as well as dorsal-ventral (D/V), constitutes the mechanism that generates positional information. The opposing cues of Wnt/β-catenin signalling and Wnt inhibitors are the primary signal for setting up the A/P axis. BMP and its inhibitors establish the D/V axis. On the A/P axis, the cascade of molecular events initiated by Wnt signal culminates in activating the cluster of Homeobox (Hox) genes. The expression pattern of the Hox genes is known to be the embryonic positioning system that provides the coordinates for A/P patterning. The various tissues and organs of vertebrate embryos derive from 3 embryonic germ layers (primordial cell layers) – the ectoderm, endoderm and mesoderm. The programme that controls body axes formation impinges on the patterning of these germ layers in order to provide positional information for their appropriate differentiation along the orthogonal A/P and D/V axes. Notably, Wnt inhibition is a central cue for specifying the forebrain identity. However, globally, at what level the mechanism setting up the body axes intersects with the germ layer patterning machinery is poorly understood. Work in our laboratory shows that Wnt signalling and its inhibition plays a role in patterning mesoderm on A/P axis.

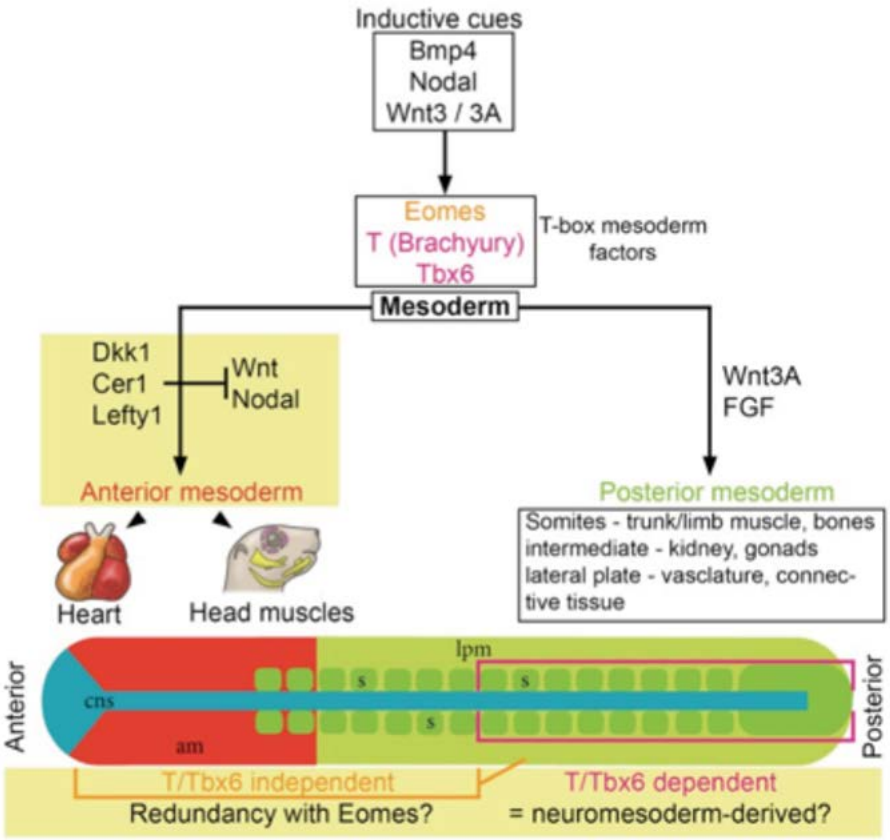

Fig 1

Patterning of mesoderm along anterior-posterior axis

Our work reveals two different levels of patterning. Mouse genetics studies highlight the two broader compartments in mesoderm based on requirement of *T / Tbx6* function for development. Our findings lead us to propose a model that this dichotomy reflects the mesodermal subtypes emerging from distinct progenitor populations (detailed in text). Within the *T / Tbx6* independent compartment, the anterior mesoderm subset that generates head muscles and heart is induced by Wnt and Nodal (TGF-β) inhibition. This finding has a broader implication: the opposing cues of Wnt signal inhibition and Wnt / β-cat signalling appears to be a conserved mechanism across bilateria to define anterior and posterior ends of the animal body axis. We propose that the Wnt cues setting up the anterior-posterior axis have a conserved role in patterning the initial anterior and posterior mesodermal subtypes. Our future work will test these ideas that have emerged from our findings.

am – anterior mesoderm; cns – central nervous system; lpm – lateral plate mesoderm; s – somites.

Our laboratory is interested in how the mesoderm is patterned along the A/P axis. Broadly, the mesoderm has two compartments, somite segment forming posterior mesoderm and the unsegmented anterior mesoderm. The anterior mesoderm comprises head skeletal muscle and heart mesoderm. Our findings show that the anterior cues i.e., Wnt inhibition may specify anterior mesoderm distinctly from posterior mesoderm (See Figure 1). Exploiting a mouse ES cell differentiation assay, we find that when exposed to a Wnt inhibitor alone or in combination with a Nodal inhibitor (another key anterior cue in mouse embryo), mouse ES-derived mesoderm gives rise to anterior mesoderm like cells by marker expression analysis. Importantly, when allowed to differentiate, this tissue generates both beating cardiomyoctes as well as skeletal muscle cells.

In parallel, we investigated the function of mesoderm specification T-box transcription factors T (Brachyury) and *Tbx6* in providing different identities to mesodermal compartments along the A/P axis. Mouse embryos mutant for either *T* or *Tbx6* display posterior segmented somite mesoderm failure; however, anterior mesoderm has no overt phenotype. We demonstrate unperturbed anterior mesoderm development in *T* as well as *Tbx6* null mouse embryos using skeletal muscle differentiation as a read out. As described in the last annual report, we have convincingly established that while posterior mesoderm requires *T / Tbx6* function, anterior mesoderm is *T / Tbx6* independent. Notably, we also find that *Tbx6* function is necessary only for the somite compartment of the posterior mesoderm. The more lateral mesoderm subtypes in the posterior region appear to develop unaffected in *Tbx6* mutants. Remarkably, however, our Cre-lox genetic tracing for *T* and *Tbx6*, reveal that both these genes are expressed in the entire mesoderm, including the anterior mesoderm. Moreover, several reports contemporary to our work

have identified key downstream anterior mesoderm genes to be targets of *T*. Therefore, while *T / Tbx6* appear to play a pan-mesodermal function, their loss of function specifically affects only the posterior somite mesodermal subtype. Taking our data together with other evidence in literature, we propose that in addition to the segmented / unsegmented mesoderm subtypes, vertebrate mesoderm could also be categorised into 1) neuromesoderm-derived somite mesoderm (posterior to mid-thorax axial level), which derives from a distinct pool of progenitors that also generates posterior spinal cord and 2) mesendoderm progenitor derived – majority of the mesoderm minus the neuromesoderm-derived somite compartment. We speculate that *T / Tbx6* dependence demarcates the neuromesoderm-derived somite mesoderm (See Figure 1). We also hypothesise that in the mesendoderm progenitor derived compartment, *T, Tbx6* and *Eomes* functionally compensate each other. *Eomes* is a T-box gene closely related to, and with similar expression domain as *T / Tbx6.* We plan to address this by two complementary approaches: A) double/triple knockout experiments of *T / Tbx6 / Eomes in vivo* as well as in embryonic stem cells in culture B) substitution mouse genetic experiments, wherein *Eomes* will be expressed under *T* promoter in *T* null background. In summary, our work has begun to reveal mechanisms at different levels in the gene regulatory programme that pattern and specify vertebrate mesoderm into distinct subtypes (See Figure 1).

Another major focus of our lab is head neural crest development. This embryonic cell type from ectoderm gives rise to cranial nerves (ectodermal derivative) as well as craniofacial skeleton (typical mesodermal derivative) and is key to vertebrate head evolution. We investigate the mechanism underlying this phenomenal trans-germ layer differentiation potential. Our comparative small RNA profiling approach has identified a number of micro RNAs (miRs) that appear to suppress neuronal ectoderm fate enriched in neural crest with mesoderm potential. We plan to dissect the role of this set of miRs. In addition, we are addressing the role of Twist1, a key mesoderm factor expressed in the neural crest, in activating the mesodermal programme in the neural crest. Together, these studies will shed light on the basis of the mesodermal character of the ectodermal neural crest.

Our future studies will investigate the conservation of developmental mechanisms that we elucidate in evolutionary developmental biology models including Amphioxus – in a comparative developmental genetics approach. This approach will help understand the evolutionary origin of the neural crest and shed light on the extent of conservation of fundamental axial patterning mechanisms that underlie animal body planning.

Honours and Awards

Ramalingaswamy-DBT fellowship, 2nd term 2016 – 2022

Invited Talks

"Distinct regulatory program governs vertebrate head mesoderm Development", NCBS Annual talks – Coming of age: Transitions in Biological systems, at NCBS, Bengaluru, India. 11 – 13th January 2016

"Vertebrate head mesoderm development", CBS, University of Mumbai – DAE, Mumbai, India. 3rd November 2015

"Vertebrate head mesoderm development is divergent from that of trunk", Indian Society of Developmental Biology - Biennial meeting at Hyderabad, India. 15 – 18th July 2015.

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8.6

Kouichi Hasegawa

UNDERSTANDING AND CONTROLLING SELF-RENEWAL AND DIFFERENTIATION IN HUMAN PLURIPOTENT STEM CELLS

The Hasegawa lab, under an instem-icems collaboration, studies the molecular mechanisms involved in self-renewal and differentiation of human pluripotent stem cells. Using defined chemical compounds, we aim to develop affordable gmp-compliant stem cell production strategies to facilitate transplantation therapies and disease modelling.

Human pluripotent stem cells (hPSCs), including embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), retain the ability to differentiate into almost all cell types in the human embryo and adult body, and to continue indefinite growth while maintaining a normal karyotype. This hPSC characteristic has great potential for application in regenerative medicine, drug development, and in the study of disease mechanisms. However, lack of knowledge about the molecular mechanisms of hPSC self-renewal and differentiation is impairing affordable stem cell production, which is required for hPSC applications, especially in transplantation therapy and drug screening. In an international and institutional collaboration with the Institute for Integrating Cell-Material Sciences (iCeMS), Kyoto University, we are focusing on understanding the molecular repertoire of hPSC self-renewal and controlling the same, using various materials including chemical compounds, polymers, nano-fibers and micro-patterning. The long-term goal of our lab is to develop disease models for high throughput molecular studies and drug screening by utilising genome-editing tools on hESCs, and patient-derived iPSCs..

hPSC self-renewal and hiPSC derivation

hPSCs can unlimitedly self-renew and can maintain their pluripotency and ability to grow in response to stimulation of signalling pathways that control growth. Most of the hPSC self-renewal conditions rely on growth factors such as bFGF and TGFβ. However, the signalling pathways stimulated by these growth factors in hPSC self-renewal are largely unknown. In addition, there are few reports on the roles of other signalling pathways in the self-renewal process. We have

previously reported that the Wnt pathway induces both hPSC proliferation and differentiation, and that DYRK signalling is involved in the differentiation induced by Wnt. Recently, in collaboration with iCeMS, we have demonstrated hPSC self-renewal independent of bFGF/TGFβ by utilising three small molecule chemical compounds, thereby generating a defined culture method for hPSC self-renewal. Additionally, the new defined culture media enhanced the efficiency of iPSC derivation from human fibroblasts. Analysis of the small molecule-mediated signalling pathways, revealed that the combination of compounds used modulated the metabolic pathways in hPSCs to favor self-renewal and iPSC derivation. We are in the process of further characterising the signalling pathways involved in the bFGF/TGFβ-dependent and -independent (our new three compound) culture conditions to understand hPSC self-renewal and iPSC derivation.

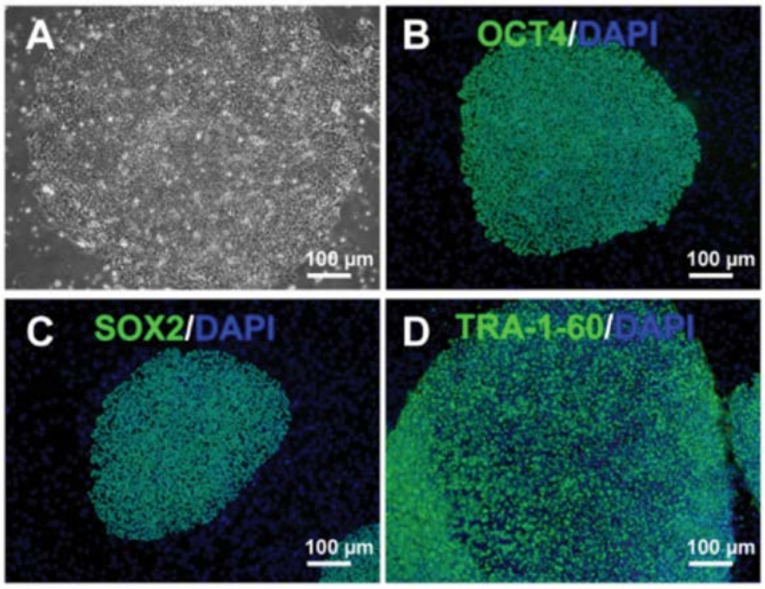

Fig 1

Derivation of iPSC lines from *P.vivax* patients. (A) Phase contrast image of a patient-derived iPSC colony. (B-D) Pluripotency markers OCT4, SOX2 and TRA-1-60 expression in patient-derived iPSC colonies. Green signals indicate immunostaining of the markers, and blue signals indicate DAPI nuclear staining.

Malaria – *P.vivax* liver-stage model

Malaria causative agent *P. vivax* can remain dormant in patient livers leading to relapses several weeks, months, or years after the primary infection and disease. The biology of such relapsing forms of vivax malaria remain unexplored because of a lack of sustainable and reliable model of the liver-stage malaria. This is a major bottleneck in developing and testing antimalarial drugs to target the liver-stage of vivax malaria. Therefore, it is highly desirable to develop uniform and reproducible liver-stage model systems that support the high *P. vivax* infection efficiency observed in patients. In collaboration with scientists at the National Institute for Malaria Research (NIMR), we are developing a reliable and sustainable malaria *P. vivax* liver-stage assay suitable for drug screening and study of malaria biology. For this, we have collected blood from *P. vivax* mono-infected patients, generated iPSCs from the blood cells, and differentiated them into hepatocytes using our newly developed efficient differentiation protocol. Using *P. vivax* sporozoites harvested in NIMR, we have developed a liver-stage model; although the infection efficiency in this model is still very low. To enhance the infection efficiency, we are currently collaborating with iCeMS to design aligned nano-fiber and micro-patterned plates so as to generate more physiologically relevant liver conditions.

Cancer biomarkers and cancer models

Utilising the similarities between cancer stem cells and hPSC-derived embryonic tissue progenitor cells, we are aiming to discover novel cancer biomarkers for diagnosis and targeted therapies.

Previously, we had identified a novel biomarker for pancreatic ductal adenocarcinoma using hPSCs. The biomarker is able to detect pancreatic cancer in pathological sections and patient serological samples. However, we recently showed that the antibody raised against this biomarker also detected cholangiocarcinoma and gallbladder cancer cells. Therefore, we are currently exploring the usability of this biomarker in other cancers. Additionally, we have started a new pilot project to develop an early-phase neuroblastoma model to understand cancer initiation and identify new biomarkers for this type of cancer.

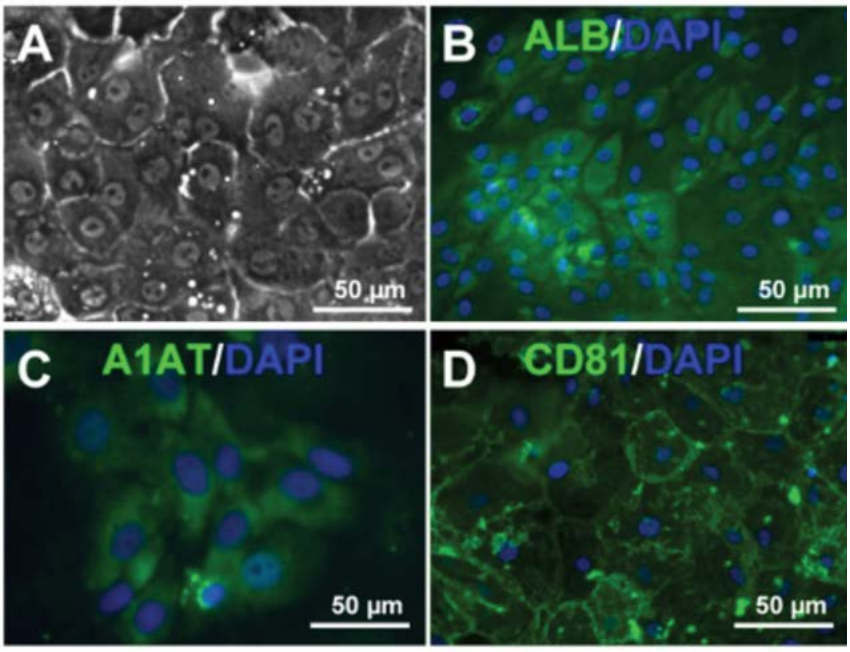

Fig 2

Hepatocyte differentiation from *P. vivax* patient-derived iPSCs. (A) Phase contrast image of hepatocytes induced from the iPSCs. (B-D) Hepatocyte marker Albumin (ALB), A1-Antitrypsin (A1AT) and host entry factor (CD81) expression in the hepatocytes. Green signals indicate immunostaining of the markers, and blue signals indicate DAPI nuclear staining.

Publication

Denham, M., Hasegawa, K., Menheniott, T., Rollo, B., Zhang, D., Hough, S., Alshawaf, A., Febbraro, F., Ighaniyan, S., Leung, J., et al. (2015). Multipotent Caudal Neural Progenitors Derived from Human Pluripotent Stem Cells That Give Rise to Lineages of the Central and Peripheral Nervous System. Stem Cells 33, 1759–1770

Invited Talks

"Growth Factor-free defined culture system for human pluripotent stem cells" at the World Stem Cell Summit 2015, Atlanta, USA. 9 – 12th December 2015

"Understanding and Controlling Pluripotent Stem Cell Self-Renewal and Differentiation" at the International Conference on Contemporary Advances of Science and Technology, and 6th India-Japan Science Seminar, Banaras Hindu University, Varanasi, India. 7 – 9th August 2015

"Understanding and Controlling Pluripotent Stem Cell Self-Renewal" at the Stem Cells and Development, Seven universities joint retreat, Shiga, Japan. 26 – 27th August 2015

"Molecular Mechanisms of Pluripotent Stem Cell Renewal and Development of Defined Culture System" at the Study Group of Cell and Regenerative Medicine 5th annual conference, Translational Research Informatics Center, Kobe, Japan. 26th July 2015

"Understanding and Controlling Pluripotent Stem Cell Self-Renewal and Differentiation" at the DANDRITE Topical Seminar at Aarhus University, Aarhus, Denmark. 18th June 2015

9

Centre for Stem Cell Research (CSCR) CMC Vellore

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

Translational Research Themes at CSCR

Three major themes for translational research have now clearly evolved:

1. GENE THERAPY

The major components of this programme involve generating an AAV vector-based gene therapy for haemophilia B in collaboration with University of Florida (UF) and Emory University (EmU), USA. This collaboration has progressed with the signing of a research agreement with UF for the production of the

vector for clinical and preclinical work with contributions from all three collaborators towards developing a novel AAV3 vector. This concept has been presented to the regulatory agencies in India for their approval. A major thrust is also on developing a programme for gene therapy for major haemoglobin disorders. Given the continuing success of the lentiviral vector approach in multiple clinical trials, this is also being pursued further along with efforts for genome-editing based approaches by disrupting the BCL11A gene to increase HbF production – all at the preclinical level at this time. Lipid base gene delivery approaches are also being explored.

This theme is coordinated by A. Srivastava and the group includes R.V. Shaji, M. Murugesan, S. Thangavel and S. Marepally.

2. MUSCULOSKELETAL REGENERATION PROGRAMME

The focus of this programme is on articular and physeal cartilage replacement, and bone and muscle regeneration in different clinical conditions. Successful implantation of autologous MSC loaded scaffolds have been done in five children with large segmental bone defects showing radiological union at 2-3 months in all patients in one of the first such studies in the world. This will be extended to 5 more patients. The group has completed a 3-year evaluation of expanded autologous chondrocytes in monolayer for physeal regeneration in children with satisfactory outcomes. They have also carried out two preclinical studies in articular cartilage repair and have tested four different scaffolds. Outcomes have shown good quality cartilage regeneration on gross evaluation. The effect of shockwaves on growth plates for modulating longitudinal bone growth is also being explored under this theme. The preliminary outcome from organ culture and in vivo studies (at 2 months) has shown that shockwaves induce cellular hypertrophy at high energy. The group is also performing various in vitro and in vivo experiments related to simulating an ideal condition to transplant satellite cells for sphincter muscle regeneration.

This area of research is coordinated by V. Madhuri.

3. APPLICATIONS OF IPSC TECHNOLOGY

Within this theme, there are two areas of translational research. The first is with regard to developing disease models using iPSCs. Apart from using them to study specific interests in disease mechanisms in Fanconi anaemia, Diamond Blackfan Syndrome and Congenital Dyserythropoietic Anaemia, this platform is also being utilised for genome editing purposes towards gene correction studies in thalassemia and sickle cell disease, and to establish a genome editing platform to complement the work being done for gene therapy. The other area of application of iPSC technology is to develop a bank of iPSCs from healthy donors who have homozygous HLA haplotypes as such individual have the possibility of being donors for cells / tissues for a large number of individuals in the community. This work is being done in collaboration with the DATRI donor registry. Blood samples from three such individuals have been collected and iPSC production has been initiated. 50-100 more samples will be collected over the next year and 10-20 iPSC cell lines of the most frequent haplotypes in India will be generated.

This work is led by D. Daniel and R.V. Shaji.

4. OTHER PROJECTS:

Apart from the major thematic research programmes, there are also several areas of project-based translational research that scientists at CSCR are pursuing. These include a study of haemoglobin gene regulation in an ex-vivo HSC based human erythropoiesis model using shRNAs and NG- based methods for RNAi screening (R.V. Shaji); a study on MSC derived exosome-based cell-free therapies (Sanjay Kumar); and studies on the molecular and morphological defects in blood vessels of type 2 Diabetes using the gestational diabetes mellitus (GDM) placenta as a model system, including analysis of the transcriptome of GDM capillary endothelium (R. Samuel). A technology platform for high-throughput screening of stem cells as well as injectable and shape memory gels for long-term, scalable culture and differentiation of stem cells under 3D culture conditions is also being developed (M. Ramalingam).

Publications

Srivastava, A., Mason, C., Wagena, E., Cuende, N., Weiss, D.J., Horwitz, E.M., and Dominici, M. (2016). Part 1: Defining unproven cellular therapies. ***Cytotherapy*** 18, 117–119

Weiss, D.J., Rasko, J.E.J., Cuende, N., Ruiz, M.A., Ho, H.-N., Nordon, R., Wilton, S., Dominici, M., and Srivastava, A. (2016). Part 2: Making the "unproven" "proven." ***Cytotherapy*** 18, 120–123

Hareendran, S., Ramakrishna, B., and Jayandharan, G.R. (2016). Synergistic inhibition of PARP-1 and NF-ĐB signalling downregulates immune response against recombinant AAV2 vectors during hepatic gene therapy. ***Eur. J. Immunol.*** 46, 154–166

Carcao, M., and Srivastava, A. (2016). Factor VIII/factor IX prophylaxis for severe haemophilia. ***Semin. Haematol.*** 53, 3–9

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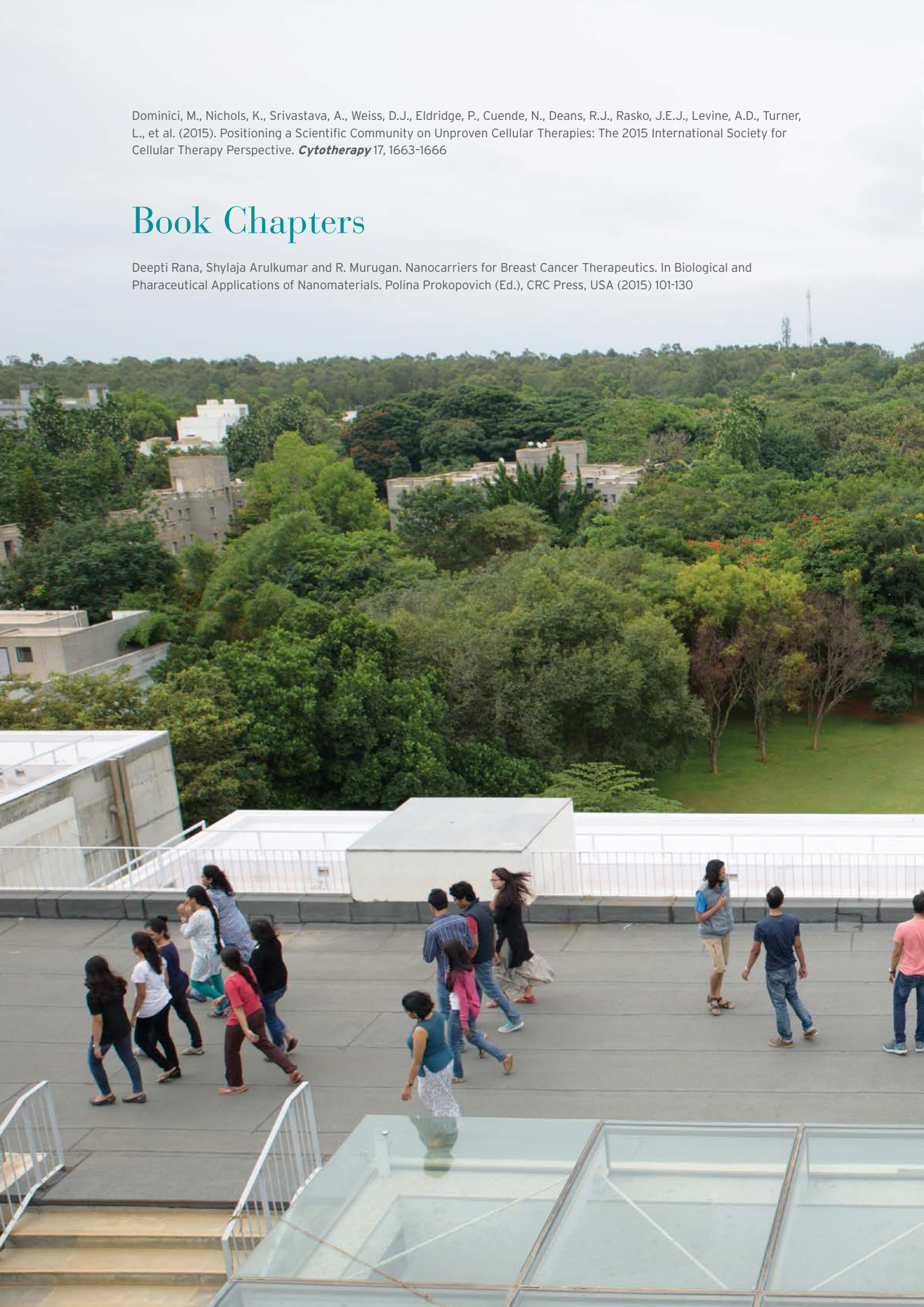


dhan@instem.res.in

10

NEW INVESTIGATOR: *Dhandapany* Perundurai

GENES, MECHANISMS AND THERAPIES FOR CARDIOMYOPATHIES

Cardiomyopathies are a group of heart muscle diseases that often lead to progressive heart failure with significant mortality. Every year about 1,000-5,000 new cases are diagnosed with cardiomyopathy, with a significant number of the patients being less than 40 years of old. The cause of a significant percentage of cardiomyopathies (~40%) remains unknown with poorly defined mechanisms and no curative therapies.

We are broadly interested in exploring new genes, mechanisms and relevant drugs that have significant clinical and curative impact on cardiomyopathies. Our group encompasses a multi-disciplinary approach involving Next Generation Sequencing in identifying new genes and the mechanistic bases of cardiomyopathies using various *Drosophila* and mouse models.

Next Generation Sequencing (NGS) to identify novel genes for cardiomyopathies

We have recently organised a total of 90 unrelated Indian cardiomyopathy patients (who are negative for reported genes) and their family members. We are performing whole exome sequencing in selected index patients with their respective family members as controls for identifying new cardiomyopathy genes.

An in vivo large-scale small molecules screening system using Drosophila model

We have generated multiple lines of transgenic Drosophila models of cardiomyopathy with different sarcomeric mutations respectively. In this objective, we will use these flies as a whole animal model to screen clinically relevant FDA approved compounds to identify preliminary novel therapeutic targets from a large set of drugs (2000 FDA drugs). The promising targets will be further tested in mice models (outlined below). Our future plans also include screening for new candidate therapeutics for cardiomyopathies from FDA-approved drugs using cardiomyocytes derived from patient-specific human induced pluripotent stem cells (hiPSCs).

Humanised transgenic mice models of cardiomyopathies

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

In addition, we have also generated a transgenic mouse model (in collaboration with Djamel, Mount Sinai) with a novel *ADIPOR1* mutation observed in familial cardiomyopathies. The mice are viable and develop cardiomyopathy around 12 weeks. The histological analyses in these mouse hearts has revealed a massive cardiomyopathy with the hallmarks of hypertrophy, including increased cell sizes and myocardial fibrosis. We will utilise these models for novel drug discoveries and studying mechanisms related to cardiomyopathies.

Publications

Akinseye, O.A., Ojike, N.I., Akinseye, L.I., **Dhandapany, P.S.,** and Pandi-Perumal, S.R. (2016). Association of Sleep Duration with Stroke in Diabetic Patients: Analysis of the National Health Interview Survey. ***Journal of Stroke and Cerebrovascular Diseases*** 25, 650-655

11

Academic Programmes

Freedom of academic inquiry is central to the success of a research environment. Young researchers-in-training, whether undergraduates, graduate students, or post-doctoral researchers, absorb the scientific culture of their host institution: the values of scientific excellence, broad exploration, academic integrity, and social responsibility. When these people leave our campus to start their independent careers, they become our ambassadors, and help to spread these fundamental values. In our 25th year, we can proudly look back at our role in training many generations of young scientists.

Our academic programmes continue to grow and strengthen, reflecting the diversity of research areas. Our PhD and Integrated-PhD Programmes are based on a structure that combines close mentorship with rigorous training. Today we offer courses and hands-on workshops in more areas than ever before: from core topics in fundamental biology, to applied areas linking science and health; from technological tools to mathematical and statistical methods; from the study of model organisms to the analysis of ecosystems and climate change. These programmes prepare our students to tackle research across the rapidly changing spectrum of the life sciences.

Our MSc Programme in Wildlife Biology and Conservation, jointly conducted by NCBS and the Centre for Wildlife Studies, continues to attract students with a passion for fieldwork and ecology. Uniquely, it combines on-campus coursework with off-campus research at field stations across the country, from the Andamans to the Western Ghats to the Himalayan foothills at Sikkim. Each student in this programme produces an original thesis, and most also publish their work as peer-reviewed papers. The alumni of this programme have gone on to careers in research, in NGOs and policy think tanks, and in Government departments.

Our post-doctoral programmes are flourishing. The Campus Fellows Programme is our flagship initiative, recruiting the best post-doctoral researchers internationally and allowing them complete freedom to design and pursue innovative research. The Simons-NCBS Fellows Programme hosts researchers with basic training in areas such as mathematics and physics, who apply their skills to tackle fundamental problems in biology. In parallel, joint post-doctoral programmes have added a new collaborative dimension. We have recruited our second batch of NCBS-inStem-Cambridge Fellows, and have instituted a similar joint programme with Institut Curie. Fellows recruited under these joint programmes combine the strengths of multiple campuses and often nucleate new lines of inquiry in their host laboratories. Our core post-doctoral programmes are growing at an unprecedented rate. Many of our post-doctoral researchers are supported by competitive extramural fellowships from sources such as AXA,

EMBO, and the Wellcome Trust-DBT India Alliance. The Campus Post-doctoral Association now has over a hundred members. It plays a key role in representing our post-doc community, and also coordinates activities such as the annual Post-doctoral Research Symposium.

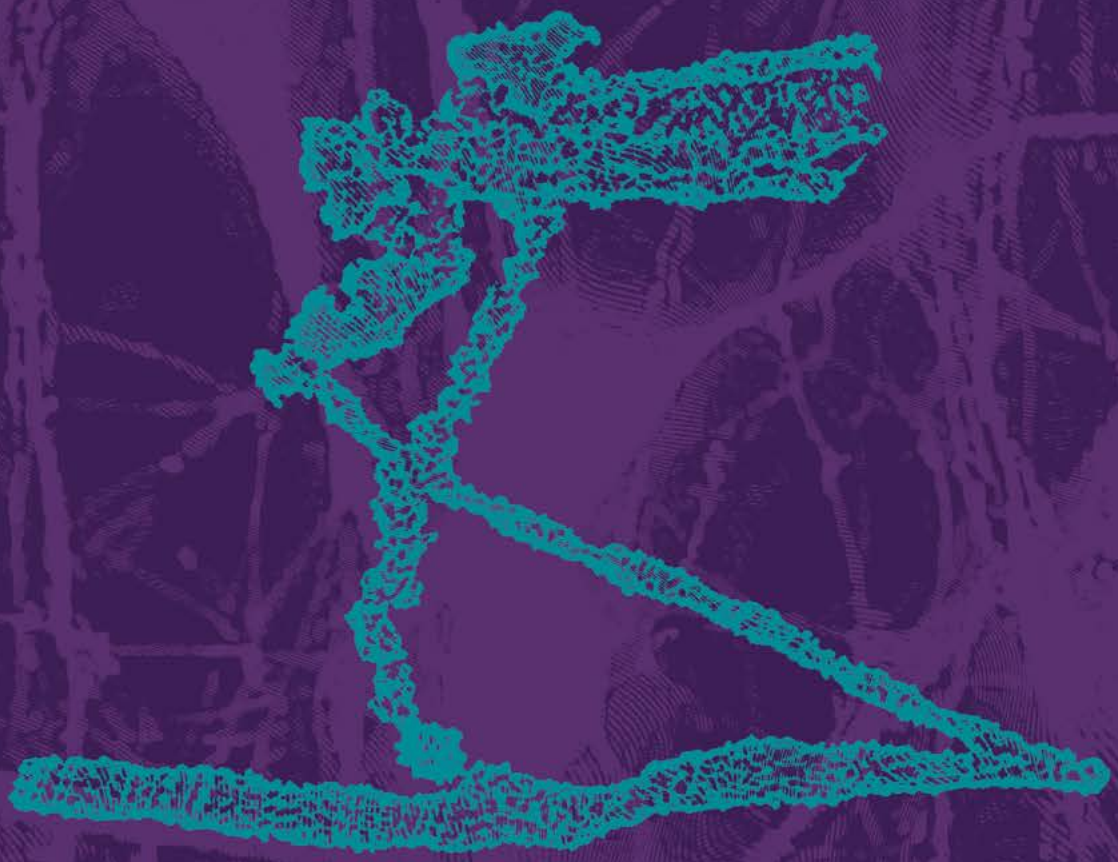
We are aware that our students are no longer restricted to a traditional academic path leading from a PhD to a post-doc to a faculty position. This is why we offer broad exposure to the modern career options that rigorous scientific training affords: science journalism and scientific writing; research opportunities in industry; the world of innovation and entrepreneurship. Students and post-docs have the opportunity to meet and interact with mentors in targeted workshops or during our annual campus-wide Career Day, so they are better prepared to make decisions about how to launch their independent careers.

Apart from our graduate students and post-doctoral researchers, large numbers of undergraduate students visit our campus for periods ranging from weeks to months, to carry out research projects. We have formal internship programmes with BITS Pilani, IISER Mohali, MSU Baroda, Manipal University, IFOM Milan, and the University of Wurzburg. We continue to host students from the Tibetan community each year, who come to us directly after graduating from high school. Our faculty members also host students from programmes such as the KVPY or the Academies' SRFP. We run workshops for undergraduates, such as the annual Monsoon School on the Physics of Life. Many undergraduate students who have spent time on our campus through these various activities often return to pursue their PhDs guided by our faculty.

Finally, our campus now operates a calendar of outreach activities throughout the year. Science Day invites school students from around the city, to interact with our graduate students and postdocs, play with hands-on exhibits, and visit our research facilities. Our Campus Post-doctoral Association runs an annual summer workshop for undergraduate students, teaching advanced topics in areas such as cell and developmental biology. The Science and Society Programme enables engagements between science and art, theatre, history, and sociology. We invite eminent scholars to speak about the implications of scientific practice, and the role of a scientist in an increasingly technological world.

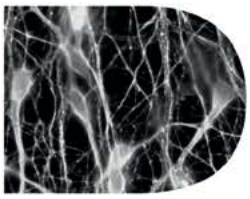
Mukund Thattai,
Head, Academic Activities

SWC



12 *Shanta Wadhwani Centre for Cardiac & Neural Research*

CNR



Established in 2012, research at the Shanta Wadhwani Centre for Cardiac and Neural Research (SWCCNR), inStem, is focused on driving new discoveries in Cardiac and Brain Biology. Under the SWCCNR, the Centre for Brain Development and Repair (CBDR) and the Centre for Cardiovascular Biology and Disease (CCBD) receive unrestricted and flexible support from the Research and Innovation (RIN) division of the Wadhwani Foundation. The support provided by the Foundation has facilitated the establishment of an international collaborative environment and in creating cutting-edge technologies to enhance the translational capabilities at SWCCNR, inStem. Examples of existing collaborations at the SWCNNR are long-term engagements with scientists and clinicians from Stanford University, University of Edinburgh and NIMHANS, which have broadened the scope of research programs and built capacity on campus. The teams at SWCNNR have been further strengthened through the addition of several young researchers and new initiatives.

The CBDR theme hosts two interdisciplinary brain research programs, the Centre for Neuosynaptopathies (CNS) and the Accelerator Program for Discovery in Brain Disorders using Stem cells (ADBS). The team of researchers at CNS – Sumantra Chattarji (NCBS), Ravi Muddashetty (inStem) and Biju Vishwanath (NIMHANS) and Richard Morris, Peter Kind and Siddharthan Chandran (University of Edinburgh) – focus mainly on understanding the molecular and cellular events in neurodevelopmental and neurodegenerative brain disorders. The CNS program is centred around studying Autism Spectrum Disorders (ASDs) and Intellectual Disabilities (ASD/ID) whereas the focus of the ADBS program is on mental disorders. Both programs have a commitment to research, which focuses on currently unmet clinical needs. This includes the development of platforms and model systems to enable discovery-led translational studies in the field of neurological and psychiatric brain disorders. Research at CBDR is expected to aid in understanding the cellular and physiological basis of such disorders, and devising large scale screening systems, to find effective pharmaceutical compounds to treat them.

Towards the aim of stem cell based disease modelling, work at the Centre has led to the successful generation and characterisation of neuronal cell types derived from human iPSC. The development

genetically engineered rat models to study autism-related behaviours has been another major achievement. The team have also developed new behavioural assays to examine social communication between rodents using emotionally salient ultrasonic vocalization (USV) calls. These novel developments are likely to bring brain research in animal models closer to observed conditions in human patients to further our understanding of brain disorders.

The CCBD group brings together expertise in biochemistry, biophysics, genetics, structural biology, computational biology and clinical studies to understand factors controlling the power output of the human heart with a focus on cardiac myopathies. Led by James Spudich (Stanford University) and Sivaraj Sivaramakrishnan (University of Minnesota and Adjunct Faculty, inStem), the CCBD team includes John Mercer, Minhaj Sirajuddin and Dhandapany Perundurai (inStem), Kouichi Hasegawa (iCeMS and Visiting Faculty, inStem), R. Sowdhamini (NCBS) and Maneesha Inamdar (JNCASR and Adjunct Faculty, inStem). The team has successfully developed biochemical and biophysical assays to understand the activity of sarcomeric proteins such as tropomyosin and are developing additional motility assays for other motor proteins such as myosin. New initiatives at the program include the functional characterisation of human induced pluripotent stem cells (hiPSCs) from patients, and identifying new cardiomyopathy genes using exome sequencing. Another success story from the CCBD group has been the development of a technology to differentiate human embryonic stem cells into beating cardiomyocytes. This has enabled single cell analysis which will aid in the creation of a drug testing platform to identify potential drugs to treat cardiac diseases.

The long-term research goals at CCBD strive to identify common cardiac disease mechanisms that can be corrected using small molecules targeted to the contractile protein network at the core functional unit of the human heart.

These two unique programs at the SWCCNR have furthered the cardiac & neural research capabilities and have advanced the existing translational focus at inStem.

Publications

Till, S.M., Asiminas, A., Jackson, A.D., Katsanevaki, D., Barnes, S.A., Osterweil, E.K., Bear, M.F., **Chattarji, S.,** Wood, E.R., Wyllie, D.J.A., et al. (2015). Conserved hippocampal cellular pathophysiology but distinct behavioural deficits in a new rat model of FXS. ***Hum. Mol. Genet.*** 24, 5977–5984.

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13

RDO@inStem

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

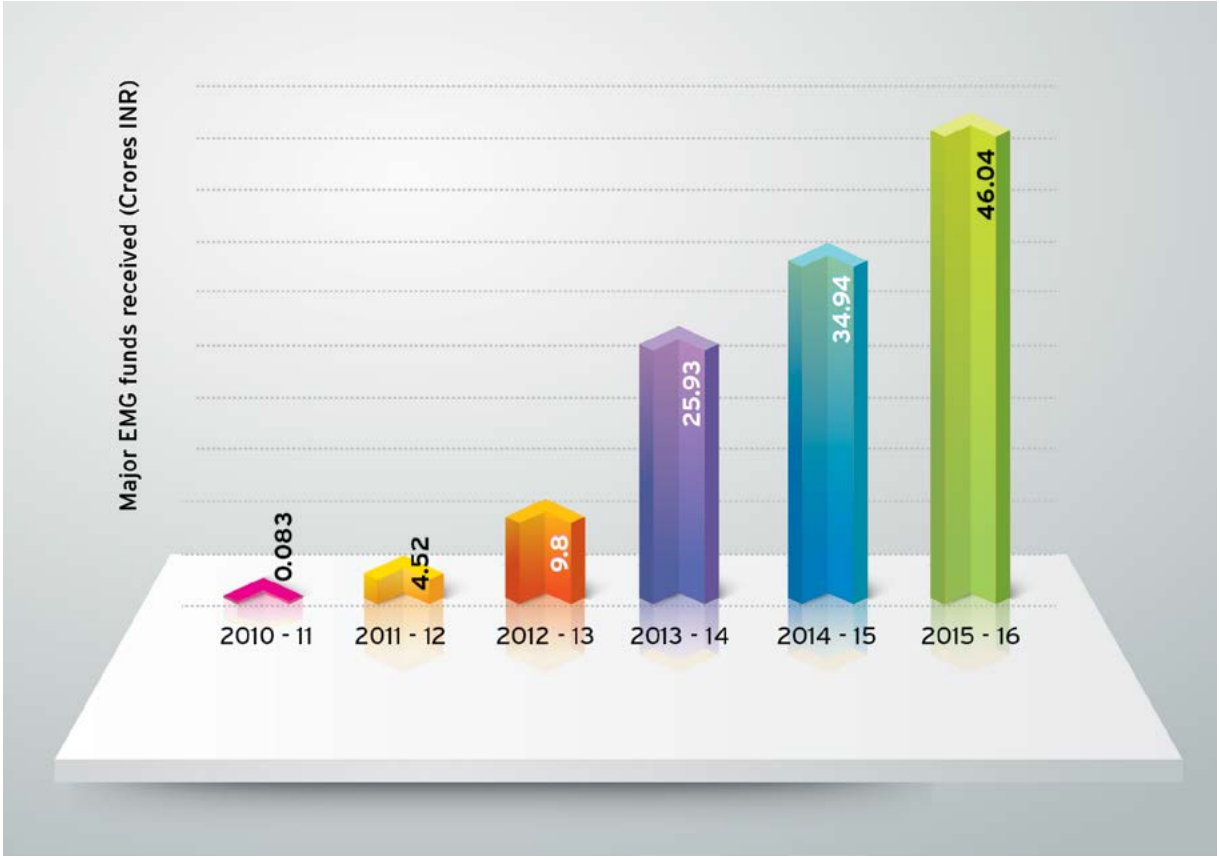
Over the course of the last six years, the Sponsored Research team within the RDO has continued supporting the diverse needs of the campus in fundraising, grants management and contract negotiation for research funding from funding agencies, corporate sources, charitable organizations and philanthropic donors. The Communications Office team within the RDO supports matters relating to print communications, graphic design, external liaison, news, press engagements and other communications. Over the course of this year, the team has created and sustained social media channels for NCBS and inStem and also for three major research programs on the campus- the Chemical Ecology program, the Simons Centre for the Study of Living Machines and the newly launched Accelerator program for Discovery in Brain Disorders using Stem cells (ADBS). More recently, the Developmental Activities team at the RDO has started working across with campus colleagues and external individuals and organizations to identify fundraising priorities, facilitate campus funding, manage donor engagement events, communications matters and build sustainable relationships with donors.

Building on previous philanthropic support from organizations such as the Wadhwani Foundation, Simons Foundation and the Tata Trust, the campus has recently invested considerable effort into developing new links to other sources of charitable funding. Earlier this year, the campus launched a bold new initiative centred around the use of stem cell technology in research, diagnostics and therapeutics. Titled "Accelerating the application of Stem cell technology in Human Disease" or ASHD, the new program is jointly supported by the Department of Biotechnology (DBT), Government of India and the Pratiksha Trust, a charitable trust setup by Infosys co-founder Mr. Kris Gopalakrishnan and his family. In parallel, the campus has now also launched an Endowment Fund, with generous corpus support from the Infosys Foundation for the creation of student travel awards.

An Endowment Fund, in addition to other existing funding support for the campus, is now essential for the Cluster to build, develop and sustain an outstanding, internationally competitive scientific hub in India, at par with the best institutions worldwide. An Endowment fund would be invaluable for us to catalyze nascent ideas, support unanticipated expenses, further the exchange of talented research investigators to and from our campus and help us engage effectively with the broader social context we work in. This is the 25th year of NCBS and we plan to initiate the campus Endowment Fund with a corpus of 25 crores in 2016 and will strive to add 25 crores each year until we reach our target of 100 crores in 2020. It is our hope that if philanthropists support our mission and value our work, the cumulative impact will be immense.

Developing the portfolio of philanthropic and other support to the campus has required sustained work from the team on all fronts, including our outreach activities. Work at the RDO is made possible by a well-knit group of dynamic and professional individuals, entrepreneurial in spirit and firmly committed to offering several key services to the campus at the boundaries of science, management and outreach. With a vibrant team, emerging opportunities on the campus and new connections on the outside, we look forward to a rewarding journey further ahead for the RDO, supporting campus research funding, communications and the Endowment Fund.

Savita Ayyar
Head, RDO



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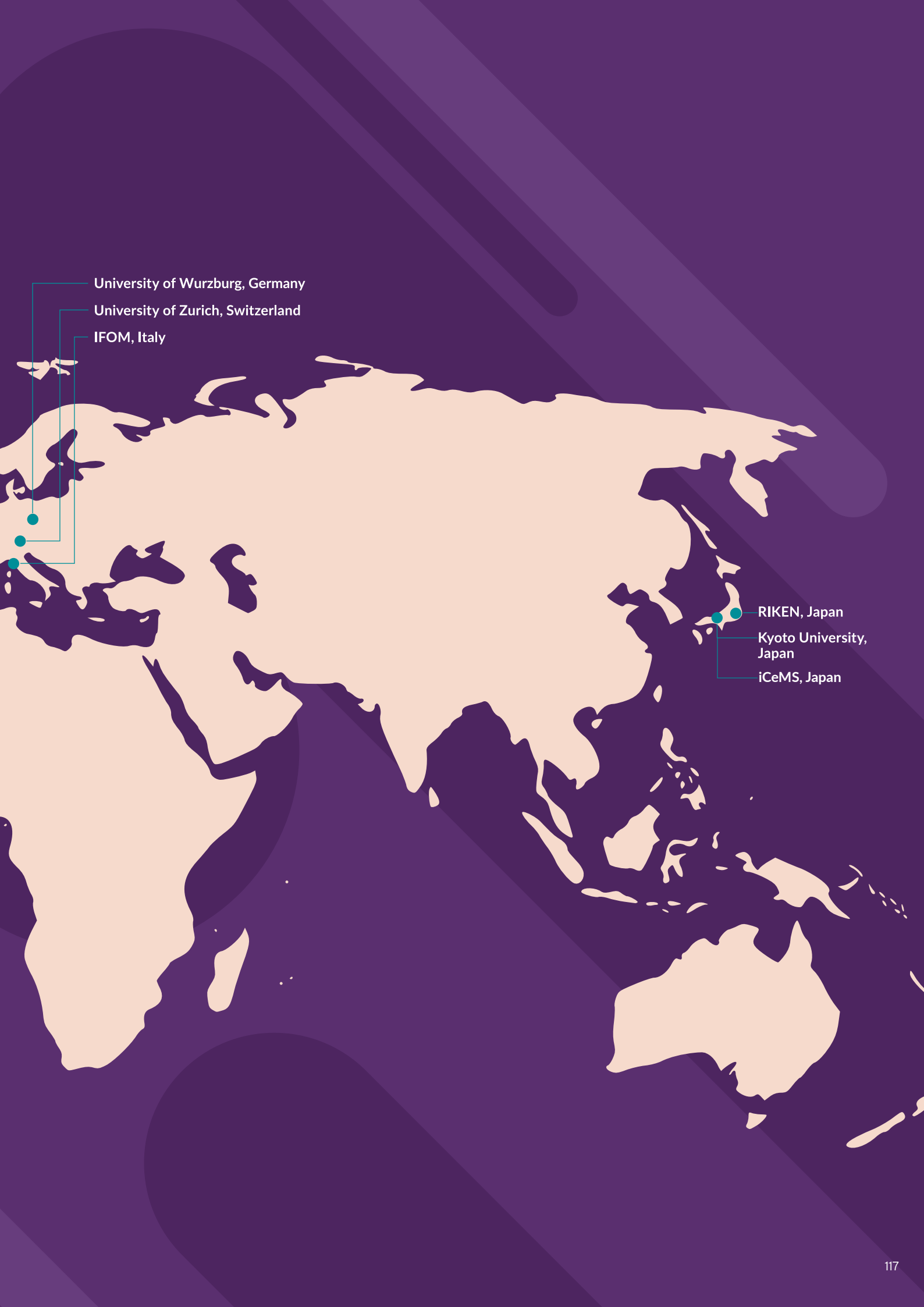
inStem International Collaborations


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

15

inStem National Collaborations

16

inStem Faculty

Core Faculty

Apurva Sarin, Senior Professor & Dean

S Ramaswamy, Senior Professor

Srikala Raghavan, Assistant Investigator

Shravanti Rampalli Deshpande, Assistant Investigator

Akash Gulyani, Assistant Investigator

Colin Jamora, Associate Investigator•

Dasaradhi Palakodeti, Research Investigator

Archana Purushotham, Research Investigator

Praveen Kumar Vemula, Research Investigator

Ravi S Muddashetty, Research Investigator

Ramkumar Sambasivan, Research Investigator

Tina Mukherjee, Assistant Investigator

Minhaj Sirajuddin, Assistant Investigator

Sunil Laxman, Assistant Investigator

Arjun Guha, Research Investigator

Dhandapany Perundurai, Assistant Investigator

Visiting Faculty

Kouichi Hasegawa, Visiting Assistant Investigator••

Kenichi Suzuki, Visiting Associate Investigator••

Jeff Abramson (UCLA), Collaborative Science Chair

James Spudich (Stanford), Collaborative Science Chair

Ashok Venkitaraman (Cambridge), Collaborative Science Chair

Siddharthan Chandran (U. Edinburgh), Collaborative Science Chair

Peter Kind (U. Edinburgh), Collaborative Science Chair

Mahendra S Rao (NYIRM, New York), Collaborative Science Chair

Sivaraj Sivaramakrishnan (U. of Minnesota), Visiting Faculty

Maneesha Inamdar (JNCASR), Adjunct Faculty

Anil Prabhakar (IIT, Madras), inStem Associate

Sanjeev Jain (NIMHANS, Bengaluru), Adjunct Faculty

Jyotsna Dhawan, (CCMB, Hyderabad), Visiting Senior Professor

Ramanuj Dasgupta (GIS, Singapore), Visiting Associate Investigator 2012-2015

John Mercer, Visiting Faculty

Azim Surani (Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK) JN Fellowship, DST, India

• In collaboration with IFOM (Milan, Italy)

•• In collaboration with iCeMs (Kyoto, Japan)

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inStem Leadership Committees

A. SOCIETY

Prof K VijayRaghavan, Secretary to the Government of India, DBT, New Delhi

Prof Satyajit Mayor, Director, NCBS & inStem, Bengaluru

Dr Alka Sharma, Director & Scientist F, DBT, New Delhi

Ms Sumita Mukherjee, JS & FA, DBT, New Delhi

Mr Chandra Prakash Goyal, Joint Secretary (Administration), DBT, New Delhi

Dr Satyajit Rath, Scientist, NII, New Delhi

Dr Kiran Mazumdar Shaw, CMD, Biocon India Ltd., Bengaluru

Dr Sunil Thomas Chandy, Director, CMC, Vellore

Prof H. Sharat Chandra, Hon. Director, Centre for Human Genetics

Prof Alok Srivastava, Head, CSCR & Professor of Medicine, CMC, Vellore

Prof K Muniyappa, Chairman, Department of Biochemistry, IISc, Bengaluru

Prof Goverdhan Mehta, Former Director, IISc & CSIR Bhatnagar Fellow, Bengaluru

Prof P Balaram, Molecular Biophysics Unit, IISc, Bengaluru

Dr Chittaranjan Yajnik, KEM Hospital, Pune

Dr Chandrima Shaha, Director, NII, New Delhi

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Prof Apurva Sarin, Dean, inStem, Bengaluru

Prof S Ramaswamy, Dean, inStem, Bengaluru

Prof Upinder S Bhalla, Dean, NCBS, Bengaluru

Mr A N Ramachandra, Head – Administration, inStem, Bengaluru

B. GOVERNING COUNCIL

Prof K VijayRaghavan, Secretary to the Government of India, DBT, New Delhi

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Ms Sumita Mukherjee, JS & FA, DBT, New Delhi

Mr Chandra Prakash Goyal, Joint Secretary (Administration), DBT, New Delhi

Dr Satyajith Rath, Staff Scientist, NII, New Delhi

Prof Chandrima Shaha, Director, NII, New Delhi

Dr Chittaranjan Yajnik, KEM Hospital, Pune

Dr Sunil Thomas Chandy, Director, CMC, Vellore

Prof K Muniyappa, Chairman, Department of Biochemistry, IISc, Bengaluru

Prof Alok Srivastava, Head, CSCR and Professor of Medicine, CMC, Vellore

Prof Jyotsna Dhawan, Visiting Senior Professor, inStem & Chief Scientist, CCMB, Hyderabad

Prof S Ramaswamy, Dean, inStem, Bengaluru

Prof Upinder S Bhalla, Dean, NCBS, Bengaluru

Prof Apurva Sarin, Dean, inStem, Bengaluru

Mr A N Ramachandra, Head – Administration, inStem, Bengaluru

C. SCIENTIFIC ADVISORY COMMITTEE

Prof Satyajit Mayor, Director, NCBS & inStem, Bengaluru

Prof Azim Surani, Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK

Prof Alejandro Sanchez Alvarado, Howard Hughes Medical Institute, USA

Prof Utpal Banerji, University of California, Los Angeles, USA

Prof Francesco Blasi, IFOM (FIRC Institute of Molecular Oncology, Milan), Italy

Prof Marco Foiani, IFOM (FIRC Institute of Molecular Oncology, Milan), Italy

Dr Satyajit Rath, National Institute of Immunology, New Delhi, India

Prof Mriganka Sur, Picower Institute for Learning and Memory, Massachusetts Institute of Technology, USA

Dr Mahendra Rao, Senior Scientific Advisor at NYSCF (New York Stem Cell Foundation)

Prof S Ramaswamy, Dean, inStem & CEO, C-CAMP, Bengaluru

Prof Jyotsna Dhawan, Visiting Senior Professor, inStem & Chief Scientist, CCMB, Hyderabad

Prof Upinder S. Bhalla, Dean, NCBS, Bengaluru

Prof Apurva Sarin, Dean, inStem, Bengaluru

D. FINANCE COMMITTEE

Prof K VijayRaghavan, Secretary to the Government of India, DBT

Prof Satyajit Mayor, Director, NCBS & inStem, Bengaluru

Dr Alka Sharma, Director & Scientist F, DBT, New Delhi

Ms Sumita Mukherjee, JS & FA, DBT, New Delhi

Prof S Ramaswamy, Dean, inStem, Bengaluru

Prof Apurva Sarin, Dean, inStem, Bengaluru

Prof Upinder S. Bhalla, Dean, NCBS, Bengaluru

Mr A N Ramachandra, Head – Administration, inStem, Bengaluru

Non-Academic Staff

E. ADMINISTRATIVE STAFF

A N Ramachandra, Head - Administration

K P Pandian, Head - Strategy

K Kunhikrishnan, Officer on Special Duty (until 31st March 2016)

K M Basavarajappa, Project Officer

Sreenath B A, Purchase Officer

Uma H R, Assistant Accounts Officer

Valsala Neyyan, Administrative Assistant

Shobha R, Clerk

Aju Krishnan, Clerk

Sunitha R, Project Assistant (Admin)

Shobha B N, Project Secretary

Archana V, Administrative Assistant

Rashi Tiwari, Manager - Academic office

F. SCIENTIFIC STAFF

Rajesh R, Engineer C (System Administrator)

Anand Kumar V, Engineer C (Electrical)

Chakrapani, Junior System Administrator

Sai Sudha, Scientist D

Deanish, Technology Manager

Rifat Naaz, Technology Manager

Pankaj, Technology Manager

Avinash Kumar Kodical, Technology Manager

Muneeswaran A, Technical Assistant

G. CONSULTANTS

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