inStem

Institute for Stem Cell Science and Regenerative Medicine

Annual Report

2018–2019

inStem

2018-19

Inauguration of inStem building – ***Atal Anusandhan Bhavan*** - February 2019 by Honorable Minister Dr. Harsh Vardhan, Ministry of Science & Technology, Ministry of Health and Family Welfare and Ministry of Earth Sciences in the presence of Secretary DBT Dr. Renu Swarup.

RESEARCH PERSONNEL

- **12** Investigators *(Two Ramalingaswamy Fellows; three Wellcome Trust DBT Indi Alliance Intermediate Fellows; One Swarnajayanti Fellowship awardee)*
- **58** Students registered for PhD
- **45** Postdoctoral fellows *(Two Wellcome Trust DBT India Alliance Early Career Fellows; 16 supported by competitive postdoctoral awards from DBT and DST)*

OUTCOMES

- **~70** Extra-mural grants; 5 Multi-institutional grants
- **50** publications
- **4** patents filed
- **4** technologies developed
- **1** start-up from inStem laboratories

AWARDS & RECOGNITION

- **Praveen Vemula,** inStem: Gandhian Young Technological Innovation Award, 2019. BIRAC-SRISTI *(Biotechnology Industry Research Assistance - Society for Research and Initiatives for Sustainable Technologies and Institutions).*
- **RV Shaji,** CSCR, CMC Vellore: Wellcome-DBT India Alliance Senior Research Fellowship, 2019.

PARTNERSHIPS

OUTREACH

- **Science Day,** November 2018: more than 1000 School and college students visit the Bangalore Life Science Cluster laboratories for interactive displays.
- **Outreach event** for School students, 11 October 2019: 950 school kids participated. *https://www.instem.res.in/content/outreach-event-school-students-instem-bengaluru*
- **Outreach event** for college students, 23 October 2019: 200 college students visited the campus for a half day event. *https://www.instem.res.in/content/outreach-event-college-students-instem-bengaluru*
- Participation in **India International Science Festival,** November 2018.
- **BLiSC Science Cafe:** bringing scientists to the public in informal settings/ social venues – Four investigators from inStem have participated.
- **Jigyasa Project, Mandram & BLiSC:** communicating research in the vernacular *(Tamil, Kannada initially).*

ADVANCED SKILLING (Training through workshops)

- **iPSC training workshops:** ~70 trained
  Indo Japan ADBS Programme in Bangalore and Kyoto, Japan; NAHD Programme, CSCR, CMC Vellore.
- **ADBS-IBAB, Bangalore**
  Bioinformatics Workshop: 20 participants
- **Laboratory Internships:** year round programme for undergraduate students to spend time in laboratories from periods ranging from 1 month – 1year: 40

NATIONAL FACILITIES in partnerships at the Bangalore Life Science Cluster *(NCBS, inStem and C-CAMP):*

- **Cryo Electron Microscope facility**, inaugurated in 2018 by Bharat Ratna Prof. CNR Rao. 60 users trained and 20 external users assisted with data collection in one year.
- **National Mouse Resource:** 68 external users trained.

inStem

Pioneering a model for translational research in biology

Content

1

Director's Note

It has been an eventful time for inStem, which heralded the start of the new year with full-fledged activation *(despite the usual teething troubles!)* of its new laboratory spaces and inauguration of the new building. The new year also brought a change in leadership. inStem acknowledges with enormous gratitude the leadership of Professor Satyajit *(Jitu)* Mayor, Centre Director, NCBS, TIFR *(2013 onwards)* and inStem Director from 2013-2018, who has been pivotal in the establishment of inStem's research structures. We thank Jitu for his visionary and steadfast leadership that steered inStem through a crucial phase of its growth, including the transition into Institution-mode and the construction of the inStem laboratory building. Today, with the thematic structure embedded in inStem, we look forward to realising the investments of the previous years, as our activities mature and gain momentum and the themes hone their distinct identities.

As in previous years, this year too has seen its share of changes. More recently, one of inStem's founding deans, S Ramaswamy (Rams), also co-founder and first CEO of C-CAMP, moved to a prestigious leadership position at the Bindley Bioscience Centre at Purdue University. We congratulate Rams on this recognition and wish him the very best in this new appointment. That said, we look forward to his continued association, albeit in a slightly different *avatar*, with inStem. I take this opportunity to welcome Mr. Pawan Pahwa as the full time Head, Administration and Finance at inStem. We wish Pawan every success and offer our enduring support, as he puts robust administrative structures in place to build a responsive and well-knit administration. The services of Mr. B.S. Nagaraja *(formerly Dy. Commissioner KVS)*, who held charge as head administration since September 2017, are gratefully acknowledged.

We look forward to welcoming Arvind Ramanathan, who will undoubtedly bring new energy to the Regulation of Cell Fate (RCF) theme with a programme on metabolic regulation of tissue homeostasis in the context of skeletal muscle dysfunction. Our congratulations to Dr. Alok Srivastava, who was re-appointed Head, CSCR, inStem's clinical translational group, at CMC Vellore. As this report documents, Alok and his colleagues' efforts for the first clinical trials in India for gene therapy for patients with haemophilia is temptingly close to being realised. Giving a head start to our endeavours to drive discoveries in fundamental science to translation, we are delighted to announce the first start-up from Praveen Vemula's group at inStem. Praveen, now an Associate Investigator at inStem, and his colleagues were also recognised by the Gandhian Young Technological Innovation Award – 2019 by BIRAC-SRISTI earlier this year.

On behalf of all my colleagues at inStem, I express our sincerest appreciation to Kiran Mazumdar-Shaw *(Chairperson & Managing Director, Biocon)*, Kris Gopalakrishnan *(Co-founder, Pratiksha Trust)* and TT Jagannathan *(Chairman, TTK Prestige)* for their generous support of our activities and the continuing engagement with our campus. Their support has been truly catalytic in many spheres of our activities, apart from making an enormous difference to the opportunities we offer our younger colleagues and we are extremely grateful!

With the promise of new positions to grow our community, our brand new laboratories, and the sustained, enabling partnership with NCBS and C-CAMP in the Bangalore Life Science Cluster, inStem is poised to grow and chart new directions in fulfilment of its charter, to take discoveries in differentiation, tissue formation and regeneration, to application in frontier areas in regenerative medicine.

Apurva Sarin
Director, inStem

2

Administration Report

The institute has completed its tenth year in its pursuit for excellence in stem cell research and allied areas. Following the approval of the Revised Cost Estimate (RCE-II) in February 2017 by the Department of Biotechnology, Government of India, the infrastructure development has resumed and the entire project is expected to be completed by October 2019.

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

CSCR's *(a Centre of the Institute situated in Vellore)* accounts are integrated into the accounts of the institute for the year. The table below indicates the status of grants received and the manpower count at the end of 31-03-2019.

DETAILS	2017-18	2018-19
Core grants received	₹ 825.00 million	₹ 820.70 million
EMG grants received	₹ 457.78 million	₹ 150.82 million
No. of active grants	56	59
Manpower (including contractual and outsourced)	258	249

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

With the sanction of regular positions and the availability of its own building, the institute's functioning will be much smoother and its contributions will increase considerably in

the years to come. The RCE status has closed w.e.f.31.03.2018 and regular budgetary sanctions have been received for 2018-19 and 2019-20. A tentative budget of Rs 835.70 million was sanctioned for 2018-19 and Rs 667 million has been sanctioned for 2019-20.

Prof. Apurva Sarin took over as Director of inStem w.e.f. 01.01.2019. The newly constructed inStem building has been inaugurated by Dr. Harsh Vardhan, Hon'ble Minister for Science and Technology; Environment, Forest and Climate Change and Earth Sciences, Govt. of India on 24th Feb 2019, in the presence of Dr. Renu Swarup, Secretary Department of Biotechnology (DBT) GoI. The Hon'ble Minister marked the occasion by planting a tree and naming the building as *Atal Anusandhan Bhawan.*

Pawan Pahwa

Shri Pawan Kumar Pahwa has taken over charge as Chief Administrative Officer at inStem w.e.f. 27.12.2018.

3

inStem's Research Mandate

The Institute for Stem Cell Science and Regenerative Medicine (inStem) is India's first stem cell institute committed to understanding the role of stem cells in health and disease, with an allied translational unit, the Centre for Stem Cell Research, at CMC in Vellore.

Under a major initiative of the Department of Biotechnology (DBT) to connect institutes together, inStem, the National Centre for Biological Sciences (NCBS), and the Centre for Cellular & Molecular Platforms (C-CAMP) partnered to form the Bangalore Life Science Cluster (BLiSC), an integrated campus for activities in fundamental research (NCBS), thematic research with translational focus (inStem) and a potential for technology development and entrepreneurship (C-CAMP).

inStem's mandate is to address complex problems in areas of directed differentiation and tissue regeneration with disease relevance through collaborative research programmes involving interdisciplinary teams with translation emphasis.

Our efforts have focused on accelerating advances in stem cell biology and regeneration in the context of brain, blood and cardiac disorders, skin diseases, as well as manipulation of stem cells for iPSC-based disease and tissue modelling.

inStem has engaged with various international universities/institutes to enhance its technical and scientific capabilities. Significant amongst these have been clinical partnerships both locally and internationally, which add a strong translational component to inStem.

4

RCF

Regulation of Cell Fate

The activities of the Regulation of Cell Fate (RCF) theme are organised around the central question of how endogenous metabolites control decisions of cell fate. Metabolic reprogramming is pivotal to cell fate decisions underlying growth, proliferation, differentiation etc. The metabolic state of a cell is dependent upon both, overall nutrient availability, as well as the environmental niches a cell is present in. However, interactions between signal transduction networks and the metabolic state's that culminate in robust cellular behaviors at the scale of tissues and whole organisms, remain poorly understood. Research in the RCF theme, aims to uncover mechanisms by which metabolites control cell fate across biological systems by pursuing the following tracks:

(i) cell autonomous, (ii) spatially organised 3D organoids, (iii) in vivo model systems that integrate tissue injury and repair and (iv) physiology and hematopoiesis.

Theme Coordinator:
Apurva Sarin

4.1

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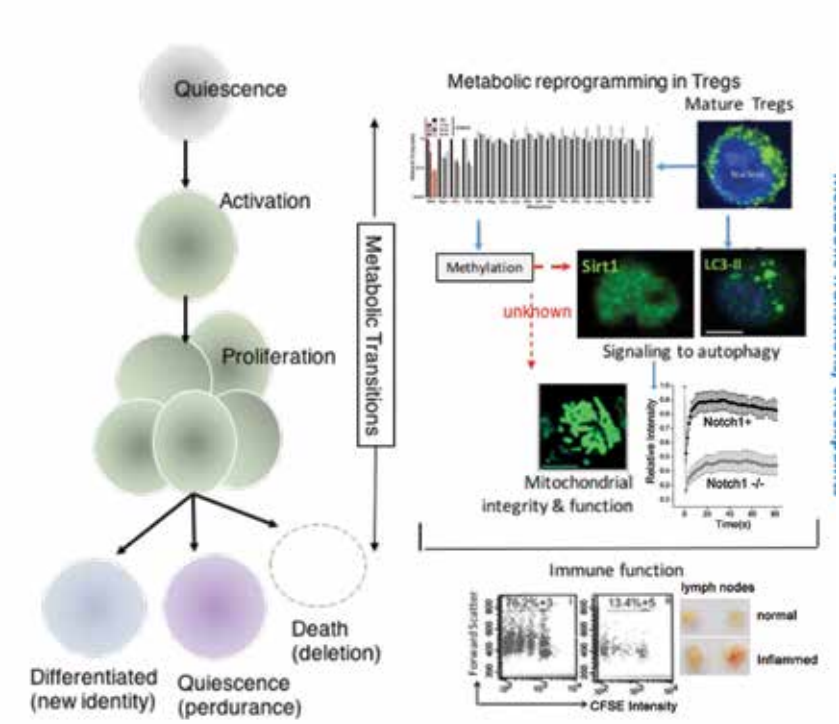
Cell Autonomous (Metabolic) Signaling Underlying Cell Fate Decisions

Cells rely on tightly regulated metabolic processes to maintain overall homeostasis. T-cell differentiation is characterised by dramatic changes in their metabolism, which is linked to the acquisition of functionality. Our work addresses the cross-talk between signal transduction networks and the maintenance of cell (metabolic) identity.

The ability of cells to sense and integrate cues and reset metabolic programmes is important for cell fate determination and lineage stability. While there is evidence in the T-cell lineage of distinct metabolism, key metabolic as well as mechanistic events that control distinct identities and cell fates remain unclear. In collaboration with Sunil Laxman we are attempting to understand metabolic cues that govern T-cell function. In the past year, our experiments have focused on metabolic reprogramming during T-regulatory cell maturation. Metabolic pathways control cellular bioenergetics, tune signaling outputs, regulate transcriptional and translational programmes, which collectively determine the final identity or fate of the cell. Our work tests the hypothesis that the orchestrated activation and inactivation of metabolic circuits underlies cell fate decisions of renewal, plasticity, differentiation and associated bioenergetics requirements.

Dependence upon defined cytokine cues for survival is a common feature of T-cells. We are interested in signalling events controlled by nutrient cues and metabolic changes that regulate immune function, in physiologically relevant contexts. In earlier work we had established a requirement for non-canonical Notch-1 signaling in the modulation of T-regulatory cell (Treg) survival and function. Using this experimental system with tunable Notch signaling we demonstrated a critical requirement for autophagy and the modulation of specific nutrient-sensing acetylases for Treg maturation *(schematic)*. The integration of signalling hierarchies with metabolic reprogramming and cellular bioenergetics in the T-cell lineage, in particular the adaptation to activating environments is an area that is not well understood. Unbiased intracellular metabolite analysis coupled with molecular and biochemical analysis identified the uptake and utilisation of sulfur amino acids as a rate-limiting step in Treg cell survival. Subsequent experiments, tracking Treg survival, established that methionine utilisation *(by the inclusion of a non-functional analogs)*, as well as its uptake *(by inhibiting transporters)*, were required for Tregs to adapt to nutrient deprivation triggered by cytokine withdrawal. Surprisingly, our experiments revealed a



The schematic summarises key observations from our work related to metabolic reprogramming in T-regulatory cells. More recent experiments using intracellular metabolite analysis, have identified a requirement for specific amino acids in T-regulatory cell maturation. How the changes in amino acid utilisation connect with subsequent signaling events and cellular responses remain to be investigated.

critical role for Notch-1 activity in amino acid utilisation. This interaction was extended to and independently confirmed in experiments in mammalian cell lines where sulfur amino acid and consequently methyltransferase regulation of Notch1 mediated anti-apoptotic activity, identified a methyltransferase as a key intermediate. The integration of Notch1 signalling with metabolic reprogramming revealed through our experiments motivates ongoing efforts, aimed at delineating Notch-1 regulated signalling in the context of nutrient and metabolic changes in the regulation of cell identity and immune function.

4.2

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The Logic of Metabolism, Metabolic Sensing, and Cell Fate Regulation

My lab is interested in understanding the logic of metabolic information flow within cells, the organising principles of metabolic networks, and how that regulates cell fates. We adopt an integrative approach to address: (i) how metabolites function as signalling molecules; (ii) how cells sense and switch metabolic states; and (iii) how metabolism controls phenotypic heterogeneity spatially and temporally in genetically identical cells.

Metabolism is incredibly dynamic, yet functions with predictable rules and constraints that are still not fully defined. Importantly, the metabolic state of a cell can causally control different cellular outcomes. My lab is interested in deciphering the organisational principles, and logic of metabolic information flow within cells. The questions we address include:

(i) What makes some metabolites special in controlling cell fates?;

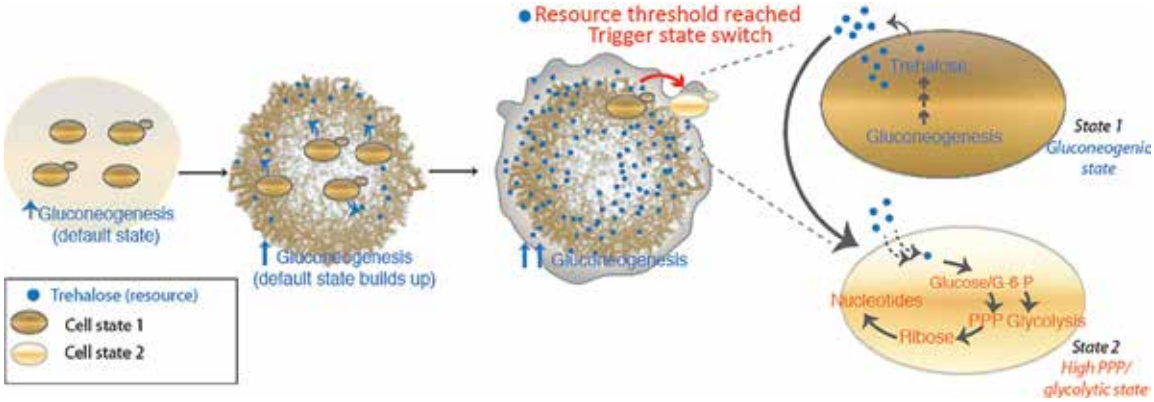
(ii) What molecular machines sense metabolic states, and how do they mediate information transfer to regulate homeostasis?; and

(iii) What is the metabolic basis for the emergence of phenotypic heterogeneity in isogenic cells?

We are especially interested in the versatile cellular functions of amino acids. These function include metabolic roles, their central importance for protein synthesis, and also as signalling molecules. However, a comprehensive understanding of these versatile roles for amino acids remains absent. We utilise a tractable model system, S. cerevisiae, to uncover universal processes of metabolic sensing and regulation, addressing the questions listed above. Our approaches are at a 'systems' level, as well as a molecular, mechanistic level. These studies in yeast inform efforts in more complex, specialised systems in mammalian cells, and have strong implications for synthetic biology and uncovering mechanisms of human diseases.

Studies initiated over three years ago have now led to many novel discoveries. Through these, we have made significant contributions in all the three areas identified above. While each study emphasised any one area, it also connects to any other two. Some of these are summarised below.

In the space of metabolites as signalling molecules, in recent work *(Walvekar A et al, MBoC 2018),* we addressed why methionine is a potent growth signal, by deciphering an anabolic program in cells that is induced by this amino acid. Continuing studies now identify mechanistic, regulatory nodes through which methionine executes this anabolic program.



The image depicts how cells in a default metabolic state can build up a resource to a threshold, which can then allow some cells to switch to a new, resource consuming state. This creates a self-organising system, where cells with different states spatially arrange. Adapted from Varahan S. et al eLife 2019.

In a distinct study, working closely with Sandeep Krishna (NCBS), we built a parsimonious mathematical/physical model explaining how threshold amounts of acetyl-CoA determine how cells oscillate between growth and quiescent states *(Krishna & Laxman, MBoC 2018).* These studies exemplify how metabolites function as fate controlling molecules, explaining how cells commit to a 'growth' state. We have also discovered novel metabolic signalling/sensing regulators. In recent work *(Gupta R et al, eLife 2019)*, we uncover how a methionine-dependent tRNA modification acts as a novel regulator of metabolic homeostasis. Here, for the first time we show how a component of the translation machine, in this case a modified tRNA, regulates metabolic *(and therefore growth)* outputs, in tune with amino acid availability. Ongoing studies in the lab are identifying novel, conserved nutrient sensing and signalling machinery. Finally, using a 'simple' system of a yeast colony, we are now establishing bio-chemical and physical rules through which phenotypically heterogeneous cells emerge in a clonal population *(Varahan S et al eLife 2019)*. Here, we propose that metabolic constraints determine how cell groups emerge and spatially organise. Collectively, we have embarked on exciting, directions, to advance our fundamental understanding of metabolism and metabolic signalling.

Further, our laboratory has built and established new resources in quantitative metabolic flux analysis *(eg. Walvekar et al, WoR 2018),* which expand our capabilities. We continue to build strengths in genomics/transcriptomics, beyond our traditional expertise in mechanistic biochemistry and cell biology. Strong ongoing collaborations have developed with Sandeep Krishna and Aswin Seshasayee *(NCBS)*, Prof. Teymuras Kurzchalia *(MPI-CBG, Dresden)*, and others. Our studies of amino acid controlled responses in yeast now inform an exciting, growing collaborative effort led by Apurva Sarin, addressing how specific metabolic components regulate cell fate in T-cells. This effort has gathered momentum with jointly mentored trainees, and growing collaborative, interdisciplinary efforts within the RCF theme.

4.3

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Systemic and Metabolic Control of Blood Development

Metabolic control of diverse biological processes forms the central focus of our theme "Regulation of Cell Fate". Allied to this, our research group aims to define metabolic demands underlying hematopoiesis. By employing genetic and genome-wide approaches we seek to identify long-range, neuronally derived systemic metabolites in blood development and the underlying physiology driving this cross-talk.

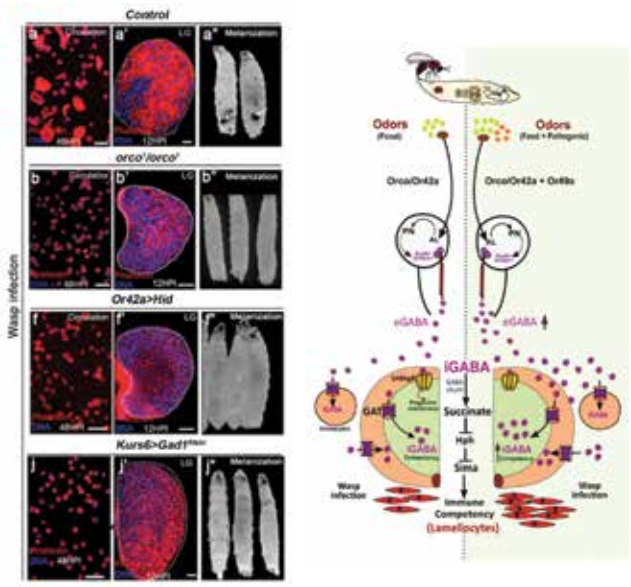
Sensory control of immunity: the importance of odour-sensing in priming immune competency

The olfactory system is a unique sensory modality that is tuned to promote animal survival by detecting odours to discriminate between favourable and unfavourable conditions. While our understanding of olfaction and its role in survival is limited to initiating behavioural changes, we find that olfaction is necessary for the establishment of a competent immune system where by odour-derived cues establish the development of a blood cell lineage termed lamellocyte, which is necessary to combat parasitic wasp-immune infections. Ongoing work reveals an unexpected association of exposure to environmental odours and their capacity to influence cellular immune responses. Specifically, larval odour-detection mediated release of neuronal GABA is necessary for generation of lamellocytes *(Figure)*. The systemic GABA is internalised by blood progenitor-cells and metabolised through the GABA-shunt pathway to succinate which prevents degradation of HIFα (Sima) protein, a potential transcription factor. HIFα is necessary and sufficient for lamellocyte induction. Limited GABA availability during larval development restricts blood-progenitor HIFα levels and consequently the lamellocyte induction potential. Unexpectedly, preconditioning *Drosophila* larvae in odour-conditions mimicking parasitoid-threatened environment, raises systemic GABA availability that further elevates blood-progenitor HIFα levels. Subsequently, infection responses in them are rapid and efficient. Overall, this study unravels the adaptive influence of environmental odour-experience on myeloid-progenitor metabolism and immune-potential, the relevance of which may be broadly conserved.

Systemic metabolic homeostasis by immune cells

As we explore the influence of metabolic pathways and key metabolites in blood development, our laboratory also explores non-immune functions of blood cells with specific emphasis on systemic metabolic homeostasis and animal growth control. To this end we pursue the following lines of inquiry:

Developmental regulation of cellular immunity by odours



Drosophila larvae spend most of their time dwelling in food. The odours derived from this eco-system defines an integral immune-component during haematopoiesis. Sensing food via Or42a stimulates projection neurons (PN) leading to downstream activation of Kurs6+GABA+ neurosecretory cells, which mediate release of GABA (eGABA) into the haemolymph. eGABA is internalised by blood cells via GABA-transporter (Gat) and its subsequent intracellular metabolism through the GABA-shunt pathway leads to stabilisation of Sima protein in them and this establishes their immune-competency to differentiate into lamellocytes. Physiologically, this sensory odour axis is co-opted to detect environmental pathogenic wasp-odours. Upon detection of wasps via Or49a in the preconditioned media (WOF), the combinatorial stimulation of both Or42a and Or49a, elevates neuronal GABA release, leading to increase blood cell iGABA mediated Sima expression. This developmentally establishes superior immune-competency to withstand the immune-challenge.

Lipid uptake by circulating myeloid-cells as a global regulator of lipid homeostasis: a role in metabolic homeostasis and animal development: Myeloid functions are dedicated to phagocytosis and immunity. Our ongoing work in *Drosophila* larval blood system has identified lipid-scavenging functions of circulating blood cells. This allows the circulating blood cells to function as regulators of global metabolic equilibrium and proper larval development. We have identified Notch (N) and Croquemort (Crq) as important effectors of this function. Perturbing them drives global changes in larvae and adults, symptomatic of metabolic disorders and enhanced sensitivity to enriched diets. Expressing lipid-scavenging receptors rescues these defects. Unlike canonical scenarios, insulin resistance (IR) and inflammation are not involved, making it a unique process to investigate. We hypothesise a model of nutrient demand by early myeloid-cells as the means to alter nutrient globally that affects overall animal physiology both immediately and in the long-term as well.

Immune metabolic homeostasis and animal growth control: *Drosophila* larvae grown on a high sucrose (25% HSD) diet develop as small adults relative to larvae grown on regular food (5% sucrose). This growth retardation has been previously shown to be a consequence of insulin resistance. We find that in D. larvae where blood cells have been ablated, when reared on HSD show further retardation of growth and much more pronounced reduction in insulin signalling. Contrary to this, larvae carrying activated macrophages as a consequence of over-expression of a constitutively activated form of PDGF-VEGF like receptor (UAS-Pvract) show recovery from the high sugar mediated growth defect and improved insulin signalling. Implying, a role for innate immune cells in growth control and dietary sugar tolerance. While insulin has been identified to play a central role in this relative slowing of growth, as apparent in Type 1 diabetes, our data highlights immune cells as additional players that work in parallel and synergistic to insulin in controlling sensitivity to signalling implicated in organismal growth axis, of which immune cell Notch function and intracellular lipid homeostasis are central players in this process.

4.4

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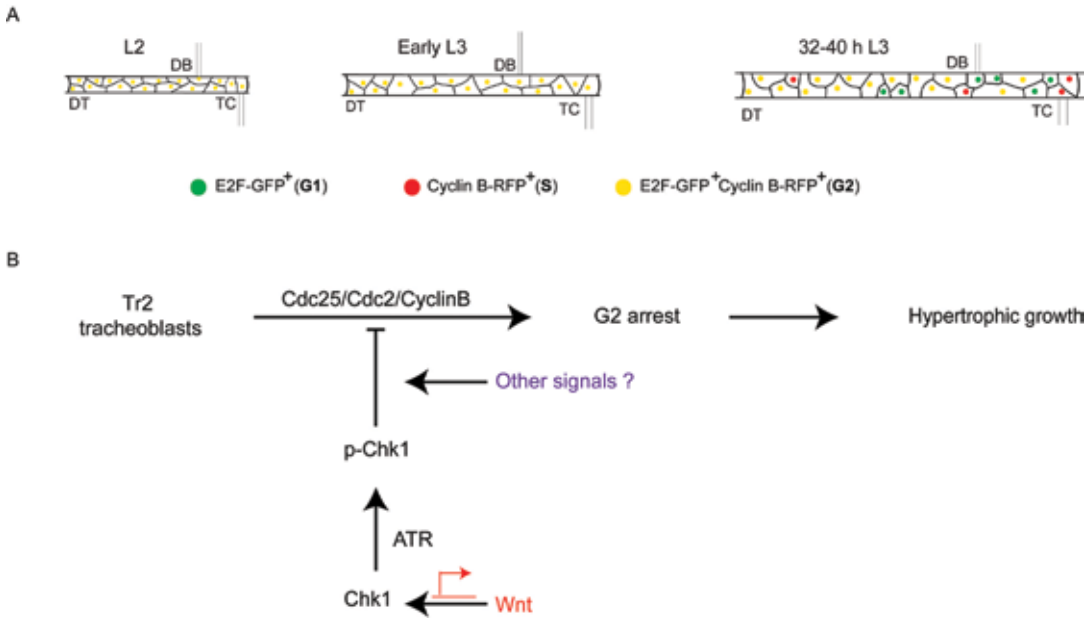
Mechanisms of Lung Injury-Repair

Our efforts are motivated by two scientific aims. First and foremost, to establish a research programme that investigates how the lung copes with damage caused by exposure to environmental toxicants. Second, to explore mechanisms underlying mitotic arrest in stem/progenitor cells with an emphasis on the regulation of G2 arrest.

Over the past year our studies on lung injury-repair have focused on the mechanisms by which the lung copes with exposure to xenobiotics that lead to oxidative and genotoxic stress. It is well known that non-ciliated, “secretory” cells of the airway epithelium an active role in the metabolism of xenobiotics that enter our bodies. Our studies in mice and in human bronchial cells lines show that these airway epithelial cells deficient in FMRP are far more susceptible to xenobiotic stress. Efforts are currently ongoing to delineate how FMRP regulates stress responses in these cells.

Our analysis of the regulation of developmental G2 arrest in progenitors of the thoracic respiratory system of adult *Drosophila* (tracheoblasts) has revealed an unusual mechanism. Tracheoblasts remain arrested for ~56 hrs. during larval stages and grow in size during this period. We find that arrested cells express the cell cycle machinery required for mitotic entry but also activate Checkpoint Kinase 1, the enzyme that mediates DNA damage-induced G2 arrest *(Kizhedathu et al, eLife 2018)*. Interestingly, arrested cells do not show elevated levels of expression of DNA damage markers nor exhibit any mitotic abnormalities upon precocious mitotic re-entry. Studies over the past year have identified Wnts as developmental signals that regulate the ATR-Chk1 axis in arrested cells and identified others that likely act downstream to Wnt to facilitate arrest *(see figure, manuscript in prep)*. Our work reinforces the idea that mitotic arrest in stem/progenitor cells is actively controlled by signals. We also find that the unusual juxtaposition of positive and negative regulators in tracheoblasts is necessary for hypertrophic growth the cells and the tracheae they comprise.

The epithelial lining of the mammalian lung exhibits low turnover during homeostasis but dramatically upregulates proliferation in response to injury. We have begun to analyse the prevalence of G2 arrested cells in adult mice and more specifically in the context of the lung. The preliminary indication is that G2 arrested cells in the adult lung are extremely rare and that most mitotically arrested progenitors are in G1/G0.



Regulation of G2 arrest in Drosophila tracheoblasts. (A) Cartoon representing the cell cycle phasing of cells in the second thoracic metamere at different larval stages. The phasing is based on expression of FUCCI reporters; cells arrested in G2 for ~56 hr express both E2F-GFP and CyclinB-RFP (yellow). Tracheoblasts grow in size during this period. (B) Model showing Wnt-dependent expression of high levels of Chk1 is necessary for G2 arrest. We also have evidence that other developmental signals are required to coordinate arrest downstream to Chk1 activation. G2 arrest in tracheoblasts in necessary for hypertrophic growth of cells and the tracheae they comprise.

Publications

A tRNA modification balances carbon and nitrogen metabolism by regulating phosphate homeostasis. eLife. 2019;8:e44795 doi: 10.7554/eLife.44795.
Gupta R, Walvekar A, Rashida Z, Liang S, Shah P, and Laxman S.

Metabolic constraints drive self-organisation of specialised cell groups. eLife. 2019;8. pii: e46735. doi: 10.7554/eLife.46735. PMID:31241462.
Varahan S, Walvekar A, Sinha V, Krishna S, and Laxman S.

Methionine coordinates a hierarchically organised anabolic programme enabling proliferation. Mol Biol Cell. 2018 Dec 15; 29(26): 3063-3200. doi: 10.1091/mbc.E18-08-0515. PMID:30354837.
Walvekar AS, Srinivasan R, Gupta R, and Laxman S.

A versatile LC-MS/MS approach for comprehensive, quantitative analysis of central metabolic pathways. Wellcome Open Res. 2018 Sep 20;3:122. doi: 10.12688/wellcomeopenres.14832.1. eCollection 2018. PMID:30345389.
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A minimal "push-pull" bistability model explains oscillations between quiescent and proliferative cell states. Mol Biol Cell. 2018 Sep 15;29(19):2243-2258. doi: 10.1091/mbc.E18-01-0017. PMID: 30044724.
Krishna S and Laxman S.

Signalling activated by nucleolar localised Notch4 Intracellular Domain underlies protection from genomic damage. bioRxiv 670588; doi: https://doi.org/10.1101/670588
Saini N and Sarin A.

Metabolic control of immune-cell competency by odours in Drosophila.
SSRN: https://ssrn.com/abstract=3382551 or http://dx.doi.org/10.2139/ssrn.338255
Sukanya Madhwal, Minkyu Shin, Manish K Joshi, Ankita Kapoor, Pirzada Mujeeb Ur Rehman, Kavan Gor, Jiwon Shim, and Tina Mukherjee.

Negative regulation of G2-M by ATR (mei-41)/Chk1(Grapes) facilitates tracheoblast growth and tracheal hypertrophy in Drosophila. eLife 2018;7:e29988.
Amrutha K, Bagul A, and Guha A.

Research Talks

Apurva Sarin:

Notch4 signaling inhibits genomic damage via interactions at the nucleolus - Indo Australian Biotechnology Conference, ACTREC Mumbai, India, October 2018.

Cellular adaptations for survival in inflammatory contexts - Workshop on Flowcytometry, Jawaharlal Nehru Centre of Advanced Sciences and Research (JNCASR), Bengaluru, India, June 2019.

Cellular adaptations for survival: Metabolic signaling underpinning cell fate decisions in T-cells - Central Drug Research Institute (CDRI), Lucknow, India, June 2019.

Sunil Laxman:

Building metabolic oscillators - 'Living Matter' - International Centre for Theoretical Sciences (ICTS), Bangalore, India, April 2018.

How to organise a structured, specialised, isogenic community: a metabolic perspective - EMBO Size and Shape, NCBS/InStem, Bangalore, India, September 2018.

Metabolic constraints determining cell fate choices and spatial organisation of cell groups - Max Plank Institute-CBG, Dresden, Germany, November 2019.

Metabolic information flow: experimental and theoretical approaches - Thirsting for Theory, International Centre for Theoretical Sciences (ICTS), Bangalore, India, June 2019.

Tina Mukherjee:

Metabolic and developmental control of hematopoiesis - New Directions in Drosophila Blood cell Biology Meeting, Vienna, Austria, September 2018.

Identification of novel neuronal signals in stem/progenitor development and maintenance - 10th Ramalingaswami Conclave, New Delhi, India, April 2019.

Arjun Guha:

Regulation of mitotic arrest in stem/progenitor cells - Indian Society for Developmental Biology Meeting, IIT Kanpur, India, December 2018.

A role for the Fragile X mental retardation protein in management of oxidative and genotoxic stress in the lung - IISER Trivandrum, India, January 2019.

Regulation of mitotic arrest in stem/progenitor cells - SPIRITS Symposium, iCEMS, Kyoto University, Japan, January 2019.

Regulation of mitotic arrest in stem/progenitor cells: Mechanisms for G2 arrest - TIFR Hyderabad, India, March 2019.

Stem cells: mysterious agents that heal our bodies - The BLiSC Science Café, MyBoTree, Bangalore, India, June 2019.

Outreach

Sunil Laxman Lab:

Outreach activities from the lab include:

(i) interactions with students from local high schools (grades 11 and 12, Biology and Biotechnology electives)

(ii) interaction with visiting school students during the campus annual Open Science Day (lab exhibits)

(iii) talks at the JN Planetarium in Bangalore (as part of the REAP programme), and policy/popular science writing in mainstream media in a personal capacity.

Arjun Guha Lab:

(i) The laboratory presented a set of interactive exhibits illustrating concepts in, “How do we breathe?” at the Science Day 2018. BLiSC, Bangalore, India.

(ii) Delivered a public lecture on “Stem cells: mysterious agents that heal our bodies” at The BLiSC Science Café, MyBoTree, June 2019, Bangalore, India.

(iii) The laboratory hosted four high school students from the Mallya Aditi International and Stonehill International Schools for a period of one week as part of a summer internship, July 2019.

5

CCBT

Centre for Chemical Biology and Therapeutics

The Centre for Chemical Biology and Therapeutics (CCBT) was established to explore innovative approaches to modulate intracellular signalling pathways disrupted in disease through a unique, integrated and multidisciplinary programme. Our first goal is to target the molecular recognition of phosphorylated proteins - a key class of protein modification vital for signalling - by specific domains. We have made strong progress in creating a unique palette of chemical probes that target signalling in this way, which will not only provide novel insights into disease mechanisms, but also help to translate this new knowledge into the discovery of novel approaches for therapy. Our work provides a framework for chemical biology and translational research across the campus.

Theme Coordinator:
Ashok Venkitaraman

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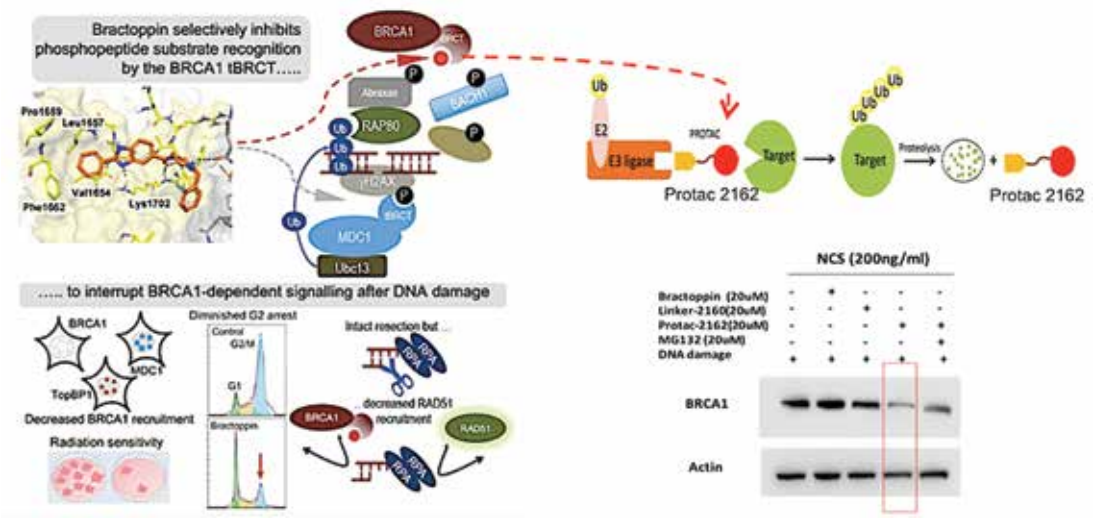
Efforts to develop new medicines over the past two decades have focused largely on creating drugs that target the active sites of enzymes like protein kinases that initiate intracellular signalling pathways. The structural similarities between the active sites of such enzyme families can make it difficult to achieve selectivity, whilst the multiplicity of substrates may lead to pleiotropic biological effects after enzyme inhibition. In this context, the emerging concept of modulating the activity of protein targets by using small molecules to inhibit regulatory domains that recognise post-translational modifications (PTMs), such as protein phosphorylation, initiated by enzymes offers important advantages allowing greater selectivity. However, such targets remain largely inaccessible to small-molecule inhibitors, posing a major impediment to chemical biology and the development of new therapies.

The CCBT has embarked on a research programme to explore new approaches to modulate intracellular signalling pathways by targeting the molecular recognition of phosphopeptide substrates via specific protein domains. Our first focus is on BRCT domains, which recognise pSer or pThr motifs using structurally distinct mechanisms. The twenty-three conserved BRCT domains found in humans participate in many different signalling pathways that control genome duplication and repair.

We have made strong progress towards this major goal. We have recently reported *(Periasamy et al., Cell Chemical Biology, 2018)* the development of Bractoppin, a first drug-like inhibitor of phosphopeptide recognition by the human BRCA1 tBRCT domain, which selectively inhibits substrate binding in vitro, and in cells, selectively blocks BRCA1-dependent signals triggered by DNA damage. Chemical matter surrounding the discovery and SAR of Bractoppin, as well as its potential uses, has been protected in a patent filing *(United States Patent Application Publication No.: US2018/0346461 A1).* We have further validated the binding mode and binding site of Bractoppin at the molecular level through site-directed mutagenesis of key interacting residues in its target, BRCA1 tBRCT, identified by molecular modeling for the future exploration of structure activity relationships. These studies *(ChemMedChem, 2019, in press)* show that Bractoppin occupies the phospho-recognising site, and two hydrophobic pockets of BRCA1 tBRCT, that are respectively common and distinct from peptide binding.

Excitingly, such insight into the binding mode of Bractoppin has enabled us to extend the conceptual foundations of our approach by structure-guided coupling of Bractoppin to an E3 ligase ligand to develop a PROTAC (Proteolysis Targeting Chimera) to induce selective target degradation. Our intimate knowledge of the structure-activity relationship (SAR) for Bractoppin has facilitated exploration of the design for a PROTAC based on Thalidomide, a well-characterised ligand for cereblon (CRBN), a substrate receptor of the cullin-4 RING E3 ligase complex. We have successfully created new PROTACs that maintain selective binding to the BRCA1 tBRCT, devising new chemical linkers that suffice to engage



ubiquitination of BRCA1 whilst avoiding any steric clashes or hindrance in tBRCT binding. Preliminary in cell experiments using these novel PROTACs already indicate a ~5-fold shift in cellular potency over Bractoppin in the suppression of BRCA1 recruitment at sites of double-strand DNA breaks. Notably, our preliminary work suggests that these novel PROTACs achieve cellular activity by inducing the degradation of the target, BRCA1.

Thus, our success in these strategies has now opened new opportunities for interrupting intracellular signalling by other members of the human tBRCT domain family, as well as other phosphopeptide-recognising domains, previously considered “undruggable”, against which we expect to create a palette of selective small-molecule leads, exemplifying an attractive new approach for enlarging the druggable proteome.

Publications

Targeting phosphopeptide recognition by the human BRCA1 tandem brct domain to interrupt BRCA1-dependent signalling. Cell chemical biology (2018).
Jayaprakash Periasamy, Vadiraj Kurdekar, Subbarao Jasti, Mamatha B Nijaguna, Sanjana Boggaram, Manjunath A Hurakadli, Dhruv Raina, Lokavya Meenakshi Kurup, Chetan Chintha, Kavyashree Manjunath, Aneesh Goyal*, Gayathri Sadasivam*, Kavitha Bharatham*, Muralidhara Padigaru*, Vijay Potluri*, and Ashok R Venkitaraman.
*Senior authors

Structure-guided synthesis and evaluation of small molecule inhibitors targeting protein-protein interactions of BRCA1 tBRCT domain. ChemMedChem (2019).
Vadiraj Kurdekar, Saranya Giridharan, Jasti Subbarao, Mamatha B Nijaguna, Jayaprakash Periasamy, Sanjana Boggarama, Amol V Shivange*, Gayathri Sadasivam*, Muralidhara Padigaru*, Vijay Potluri*, Ashok R Venkitaraman*, and Kavitha Bharatham*.
*Senior authors

Patents

Chemical matter surrounding the discovery and SAR of Bractoppin, as well as its potential uses, has been protected in a patent filing (United States Patent Application Publication No.: US2018/0346461 A1).

Awards

Best Poster award at the Society for Study of Xenobiotics annual conference (SSX India 2018) entitled: **targeting phosphopeptide recognition by the human BRCA1 tandem BRCT domain to Interrupt BRCA1-dependent signaling** presented by Dr. Jayaprakash Periasamy.

Research Talks

Life science research & its interface with engineering and allied sciences (LSRIEAS-2018) inhibitor of protein-protein interactions: HIV-1/CD80/86 for potential therapeutic interventions - BITS, Pilani, India, November 2018.
Anandi Karumbati, Team Lead, CCBT.

Interactive meeting between National Centre for Biological Sciences-TIFR, Bangalore and Bhabha Atomic Research Centre, Mumbai, India.

Parsing human disease mechanisms to develop new therapies - BARC, India, May 2019.
Gayathri Sadasivam, Lab Lead, CCBT.

Seminar on **Application of Computational Tools in Drug Discovery.**

Development of first small molecule inhibitors targetting phosphopeptide recognition by the human BRCA1 tandem BRCT domain - Advanced learning centre, Ramaiah University of Applied Sciences, Bangalore, India, January 2019.
Kavitha Bharatham, Lab Lead, CCBT.

Outreach

CCBT and IBSE (The Initiative for Biological Systems Engineering) at IIT Madras, an inter-disciplinary group dedicated to **pioneering innovative approaches and algorithms that integrate multi-dimensional data across scales collaborate to unravel biological profiles of small molecule/s modulators for novel targets** based on CCBT's proprietary small molecule chemical library.

6

CITH

Centre for Inflammation and Tissue Homeostasis

The laboratories in the Centre for Inflammation and Tissue Homeostasis (CITH) explore the emerging notion that resident immune cells in the tissue make important contributions to tissue development, homeostasis, and repair. These contributions are elegantly illustrated in an organ such as the skin whose components undergo cyclical regeneration throughout the lifetime of the animal. For instance, the epidermis of the skin turns over every few weeks and the hair follicles grow, fall out, and regrow. Additionally, the dermis of the skin as well as the subdermal fat also oscillates in volume. Intriguingly, these fluctuations occur in sync with changes in the amount and localisation of various innate and adaptive immune cells. The major activities of CITH, therefore, are focused on probing the unconventional roles of immune cells in regulating the normal cyclical regeneration of these skin compartments and its impact on the overall physiology of this organ.

Theme Coordinator:
Colin Jamora

6.1

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Wound Healing and Diseases with a "Wound Signature"

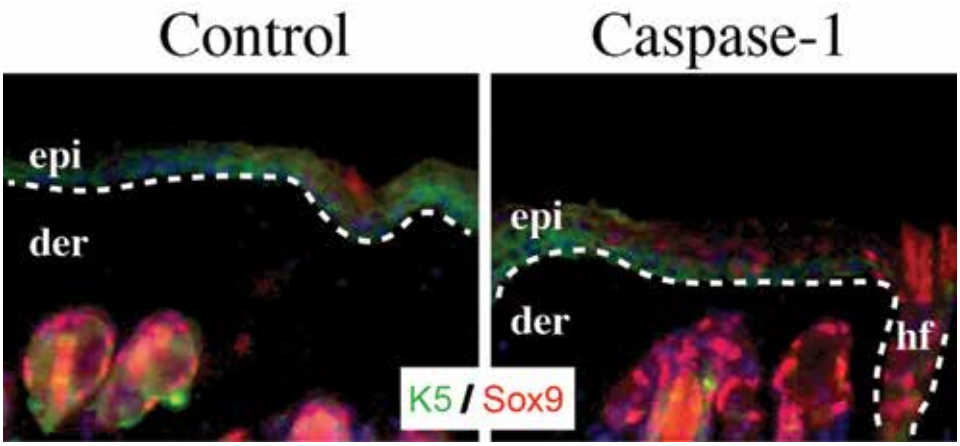
The IFOM-inStem Joint Research Laboratory directed by Colin Jamora works on deciphering the molecular mechanisms underlying wound healing. The goal is to utlise this knowledge to develop therapies for diseases where wound healing is deregulated, such as in diabetes, fibrotic diseases and cancer.

Over the past year we have made important advances in understanding how cells of the immune system play an important role in mediating the tissue repair during the wound-healing programme. Upon injury, the tissue immediately launches a self-limited inflammatory response to provide a defense against pathogens that also plays an important role in orchestrating activities that expedite recovery. However, the benefits of inflammation recede when these responses fail to resolve in a timely manner. In addition to combating potential infection from microorganisms when the skin barrier is compromised by injury, our work is contributing significantly to the emerging appreciation of the "non-immune functions of immune cells". In other words, it is becoming increasingly clear that immune cells can play an instructive role in modulating the behaviour of cells in a tissue to promote repair and regeneration. Insights from this work are providing a clearer understanding of how chronic inflammation leads to a diverse array of diseases such as fibrosis.

We recently uncovered how a protein called Plasminogen Activator Inhibitor type 1 (aka PAI-1) can cause the innate immune cell called a mast cell to infiltrate into the skin upon wounding. Mast cells are most prominently known as a mediator of allergic responses. In the context of wound healing, we found that PAI-1 can not only recruit mast cells to the skin but also mediates their interaction with the dermal fibroblasts. These dermal fibroblasts are the major source of collagen, which provides structural support for the skin and is especially important during the rebuilding of the damaged tissue. The binding of the mast cells to the fibroblasts results in their mutual activation – fibroblasts respond by increasing their production of collagen and mast cells become activated and release proteins that further promote the wound healing response *(Pincha et al., JCI 2018).*

Not only do immune cells affect neighbouring cells within the tissue, but proteins classically associated with launching an immune response are also emerging as important regulators of the wound healing programme. Recently we have found a novel wound repair function for the protein caspase-1, a critical component of a cytosolic complex known as the inflammasome that mediates the release of inflammatory cytokines from the cell. In particular, we have found that caspase-1 itself is released upon cellular stress and

unexpectedly acts as a chemokine in the extracellular milieu to attract hair follicle stem cells to the wound bed *(Ghosh et al., BioRxiv 2019, Figure)*. The recruitment of resident pools of epithelial stem cells to the wounded epidermis has long been known but the factor(s) attracting these cells to the site of damage has been a puzzle that has stumped the field for nearly 20 years. In addition to identifying a protein that can stimulate stem cells to migrate to a wound, these results open up a new perspective of why inflammatory diseases are often accompanied by epithelial hyperplasia.



Extracellular caspase-1 induces hair follicle stem cell recruitment to the epidermis. Skin explants from mice were treated with recombinant caspase-1 (Caspase-1) or vehicle control (Control). After 24 hours of treatment, skin explants were stained with Keratin-5 (K5) in green to mark the epidermis and Sox9 (in red) to mark hair follicle stem cells. The white dotted line denotes the basement membrane that separates the epidermis (epi) and hair follicle (hf) from the dermis (der).

6.2

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Epithelial Homeostasis and Inflammation: Role of Integrins, Vinculin and Macrophages

Research in the Raghavan lab focuses on understanding the role of integrins, vinculin and immune cells (macrophages), in maintaining the extracellular matrix organisation and the stem cell niche, both of which are critical for the maintenance of epithelial homeostasis.

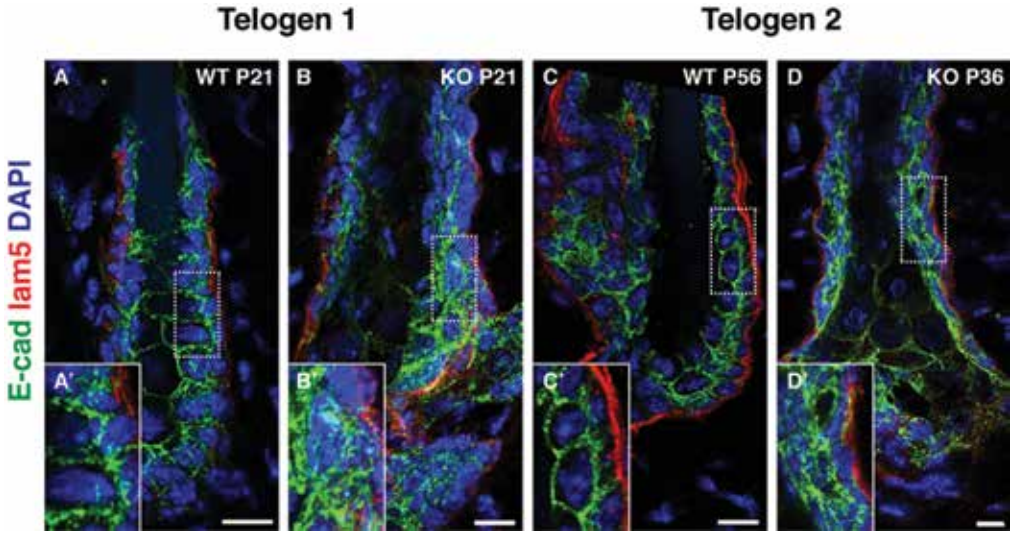
Understanding the role of vinculin in maintaining stem cell homeostasis in skin

Vinculin is a mechano-coupling, protein that is found both at cell-cell (adherens) junctions and cell-substratum (focal) adhesions. Vinculin helps to link the actin cytoskeleton to the junctions at the cell membrane. It acts as a docking protein for several focal adhesion partners and alpha-actinin at cell-cell junctions thereby regulating several signalling pathways induced by mechanical forces. In order to study the roles of vinculin in keratinocytes, we generated a skin specific conditional KO. The vinculin knockout mice displayed loss of hair, unusual size, abnormally shaped hair follicles forming cyst like structures and acceleration of the hair follicle cycle, which interestingly did not lead to complete hair loss. Label retaining experiments revealed that the hair follicle stem cells fail to maintain their quiescence in the KO, which may explain their continuous cycling. The bulge cells in the KO show defects in maintaining normal cell-cell junctions, which results in the nuclear translocation of YAP. We performed biophysical measurements in collaboration with the laboratory of Prof. Yan Jie at the Mechanobiology Institute (MBI), Singapore, and were able to show that the junctions of the vinculin KO cells exhibited reduced cell-cell junctional forces, which was rescued upon the addition of full-length vinculin to KO cells. We also observed that the phenotype of vinculin null cells was similar to the alpha-catenin null keratinocytes, thereby highlighting the role of these two proteins in maintaining stable adherens junctions. Our results suggest that the loss of cell junction stability can override the intrinsic quiescence of bulge stem cells.

Delineating the immune-epithelial cross-talk in embryonic skin

Macrophages are highly plastic subsets of leukocytes, which play a crucial role in inflammation and tissue homeostasis. Using the conditional integrin β1 knockout embryos, which serve as a very good model for embryonic sterile inflammation, we aim to understand the mechanisms underlying macrophage polarisation and its crosstalk with the epithelia in

development and disease. We depleted the skin of its macrophage populations using the CSF1R blocking antibody. The analysis of the skin revealed that upon macrophage depletion, there was not only a decrease of inflammation in the skin, but the β1 KO ECM phenotype was rescued. Interestingly, in the WT treated skin there were aberrations in epithelial differentiation and reduction in the number of hair follicles. To further elucidate the role of macrophages in regulating epidermal differentiation, we performed an NGS-based gene expression analysis of the epithelia and fibroblasts from CSF1R treated WT and KO embryos. These data will help us discern the role of macrophages in maintaining epidermal homeostasis and regulating inflammatory conditions.



Abnormal cell-cell junctions in vinculin KO bulge stem cells

6.3

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Histone Methyltransferases Guiding Development and Aging

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

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

My laboratory is investigating canonical and non-canonical mechanisms by which KMTs regulate cell plasticity in development and how perturbations of these mechanisms lead to disease states. Our quest over the past few years has identified that non-histone methylations performed by KMTs are critical for development, disease and also identified a novel role in aging. In addition, we have been successful in generating human stem cell-based tools and mouse models to investigate how histone (canonical) and non-histone proteins methylations (non-canonical) could converge to ensure the desired biological outcomes.

The following are key programmes of study in my laboratory:

Mechanisms underlying cellular plasticity regulated by KMTs

Cellular plasticity is characterised by acquisition of a new identity towards alternative fate and function. Such plasticity is an essential component of normal physiology, disease progression and therefore a major area of research interest. At the molecular level, cell state conversion must overcome several barriers including that of the epigenetic landscape in response to stimuli. While substantial efforts have been put forth to identify factors that influence cellular plasticity, a comprehensive understanding of this intriguing process still remains elusive. To get a complete overview of the cellular plasticity and its consequence on biological outcomes, we are studying KMT mediated regulation of histone and non-histone methylation during cellular reprogramming.

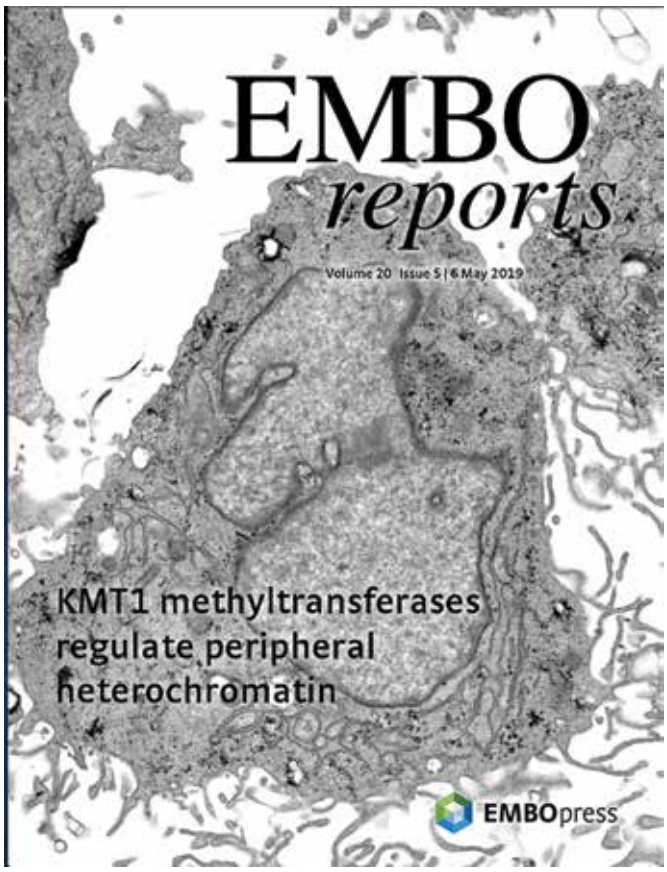
Function of KMTs in tissue repair and homeostasis

In vivo cell type conversions are commonly seen in lower organism to build lost tissue;

however, this process is much less appreciated in mammals. Recently there is a surge in the literature demonstrating such conversions in the injury-repair response to restore tissue homeostasis. A compelling example of such adaptive transdifferentiation occurs in skin injury wherein TGF-β released upon wounding converts fibroblasts to myofibroblasts. In this programme we are investigating the functions of KMTs, which are poorly defined in the context of wound healing. We are studying how EZH2 mediated H3K27 methylation regulates fibroblast plasticity during the tissue repair process.

Contribution of KMTs to aging and age-related diseases

Aging is the single most undisputable risk factor for several diseases including neuro-degenerative, metabolic, cardiovascular disease and cancers. Thus, from both the basic science and translational perspectives, intense effort has been dedicated to uncover molecular mechanisms underlying cellular aging. At the molecular level, aging leads to degenerative epigenomic changes in somatic as well as stem cell components that are responsible for the progressive loss of homeostatic and regenerative potential of a given tissue. In my laboratory we focus our studies on unconventional non-histone methylation of KMTs that contributes to the loss of cell plasticity thereby resulting in aging. Towards this we have demonstrated that the EHMT mediated H3K9 methylation and non-histone methylation of LaminB1 are critical determinants of heterochromatin organisation and its impact on fundamental changes associated with the aging process.



Research highlighted as the cover article on EMBO Reports (from Rao et al., 2019)

Publications

Methods in molecular biology. Isolating immune cells from mouse embryonic skin. 299-305. doi:10.1007/7651_2018_148
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Unraveling the ECM-immune cell crosstalk in skin diseases. Frontiers in cell and developmental biology. 7:68.
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Dynamic expression of tRNA-derived small RNAs define cellular states. EMBO Reports. pii: e47789. doi: 10.15252/embr.201947789.
Krishna S, D G Yim, V Lakshmanan, V Tirumalai, J L Koh, J E Park, J K Cheong, J L Low, M J Lim, S K Sze, P Shivaprasad, A Gulyani, S Raghavan*, D Palakodeti*, and R Dasgupta* (2019).
*co-corresponding authors

Characterisation of new variant human ES line V-H9 hESC: a tool for human stem cell and cancer research. Stem Cell Res. 37: 101444. doi: 10.1016/j.scr.2019.101444.
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PAI-1 mediates fibroblast-mast cell interactions in skin fibrosis. Journal of Clinical Investigation 128(5): 1807-1819. doi:10.1172/JCI99088
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Interactions between epidermal keratinocytes, dendritic epidermal T-cells, hair follicle stem cells. In Skin Stem Cells, 2nd Edition. Methods in Molecular Biology (Kursad Turksen, series editor). Springer Publishers
Badarinath K, Dutta A, Hegde A, Pincha N, Gund R, and Jamora C (2018).

Activation of fibroblast contractility via cell-cell interactions and soluble signals. Bioprotocols doi:10.21769/BioProtoc.3021
Pincha N, Saha D, Bhatt T, Zirmire R, and Jamora C (2018).

Research Talks

Colin Jamora:

Antimicrobial peptides in the skin - Skin Immunity Workshop, Jarkarta, Indonesia 2019.

Mechanical and Epigenetic Regulation of Wound Healing - Mechano Developmental Biology Meeting, Coorg, India 2019.

Understanding the wound healing programme and allied diseases - Indian Institute of Technology (IIT) – Hyderabad, 2019.

A non-canonical signaling pathway mediating Snail-induced fibrosis - Indo-Australian Symposium on Epithelial-Mesenchymal Transition, National Centre for Cell Science, Pune, India, 2018.

Mast Cells and fibrosis - University of Miami – Dermatology Medical Science Training Programme First Annual Conference, Miami, Florida, USA 2018.

Understanding the wound healing programme and allied diseases - IFOM-IEO Seminar Series, Milan, Italy, 2018.

Parsing the contribution of fibroblast heterogeneity to tissue fibrosis - Tata Memorial Centre – Advanced Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai, India, 2018.

Regulation of epithelial stem cells during wound healing - Manipal Academy of Higher Education – School of Regenerative Medicine. Bangalore, India, 2018.

Srikala Raghavan:

The integrin network: role of integrins in epithelial homeostasis and inflammation - Duke-NUS Medical School, Singapore, August 2018.

Role of mechano-transduction in regulating appendage size - EMBO Meeting on Size and Shape, Bangalore, September 2018.

Role of vinculin in regulating bulge stem cell quiescence - Cutaneous Biology Meeting, Brisbane, October 2018.

Role of mechano-transduction in maintaining stem cell quiescence in mouse skin - MBI 10th Anniversary Meeting, Singapore, November 2018.

Breaking barriers: role of integrins in epithelial homeostasis and sterile inflammation - Faculty of Medicine, Siriraj Hospital, Bangkok, Thailand, December 2018.

Junctional instability can override the intrinsic quiescence of stem cells - inStem Annual Talks, Bangalore, March 2019.

Breaking barriers: role of integrins in epithelial homeostasis and sterile inflammation - Japanese Society for Developmental Biology, May 2019.

Shravanti Rampalli:

Regulation of peripheral heterochromatin domain organisation via histone and non-histone protein methylation - International Human Epigenome Consortium, Hong Kong, October 2018.

Expanding the roles of histone lysine methyltransferases beyond gene regulation in reprogramming and aging - The SPIRITS International Symposium-2019 (Regulation of cell fate and disease treatment), iCMES Kyoto University Japan, January 2019. ¬

Regulation of peripheral heterochromatin domain organisation via histone and non-histone protein methylation - International Congress for Cell Biology, Hyderabad, India, January 2018.

Expanding the roles of Histone Lysine Methyltransferases beyond gene regulation in reprogramming and aging - CCMB Hyderabad, India, October 2018.

Elucidating the novel functions of KMTs in regulating cellular reprogramming - 7th Asian Chromatin Meeting, JNCASR, Bangalore, November 2018.

Modeling human disease using pluripotent cells - International Society of Gene and Cell Therapy, NIMHANS, Bangalore, India, 18-19th November 2018.

Regulation of peripheral heterochromatin domain organisation via histone and non-histone protein methylation - Genome Architecture and Cell fate Regulation Meeting, University of Hyderabad, Hyderabad, December 2018.

Outreach

Colin Jamora:

BLiSC OpenScience Day *(for elementary and high school students)*, Bangalore, India, November 2018. Designed and presented the basic cell biology of wound healing.

Lab tour and research overview for students from BMS College for Women, March 2019.

7

BDDM

Brain Development and Disease Mechanisms

Brain disorders are a global health challenge with the vast majority having no effective treatments. Despite obvious differences in their clinical presentation, many of these disorders appear to share molecular, cellular and circuit mechanisms. Our vision is to accelerate the discovery of these mechanisms and thus facilitate the delivery of effective therapeutics for these disorders.

This theme seeks to understand the development of the mammalian brain at multiple scales of organisation from molecules to brain circuits and behaviour. In particular, we are interested in exploring cell-cell interactions and sub-cellular processes that underpin normal brain development and physiology that may result, when altered, in brain diseases. Such processes include but are not limited to membrane organisation, translational control, chromatin regulation, RNA mediated mechanisms and related processes. The work within this theme seeks to link these basic biological mechanisms to aspects of human brain diseases, including disease susceptibility, disease progression, and pharmacogenomics to inform on the development of novel diagnostic and therapeutic options.

Theme Coordinator:
Sumantra Chattarji

7.1

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CNS: Centre for Neurodevelopmental Synaptopathies

Co-Principal Investigators >

Siddharthan
Chandran

Peter
Kind

Neurodevelopmental and neurodegenerative brain disorders represent a major and growing public health challenge in the developed and developing worlds. The goal of the CNS programme, has been to accelerate the discovery and delivery of effective therapeutics for neurodevelopmental disorders, specifically Autism Spectrum Disorders (ASD). At the Centre for Brain Development and Disease Mechanisms, we combine a range of expertise in several fields of neurobiology including synaptic function and plasticity, human stem cells and cognition-behaviour.

Programme 1: Stem cell biology – "Understanding autism in a dish"

Mounting evidence from recent pathological, radiological and genetic studies have shown that glia plays a pivotal role in the brain rather than being just supporting structures located around neurons. In diseased conditions, glia can be injurious or neuroprotective to neurons. How glia in ASD disease models affects overall brain function remains largely unexplored. Hence, a key focus of this programme is to examine cell autonomous versus non-autonomous effects of ASD/IDs on neurons and astrocytes.

An *in vitro* cell culture system was established to derive mature functional astrocytes following cues from developmental biology and further co-cultured with neurons to study the non-cell autonomous effects. Using whole-cell patch clamp recordings, we found that human iPSC derived neurons fire bursts of action potentials. Control (healthy) neurons co-cultured with control astrocytes exhibited low burst frequency and higher burst duration. In contrast, FXS neurons co-cultured with FXS astrocytes displayed significantly higher

frequency of bursts, but of lesser duration. Strikingly, when control neurons were co-cultured with FXS astrocytes the bursting profile of the control neurons resembled that of the FXS – with high burst frequency; and shorter burst duration. Consequently, when FXS neurons were co-cultured with control astrocytes the aberrant bursting activity was "rescued" to resemble the healthy neurons with low burst frequency and longer burst duration. Thus, the genotype of astrocytes determines the electrophysiological phenotype of neurons.

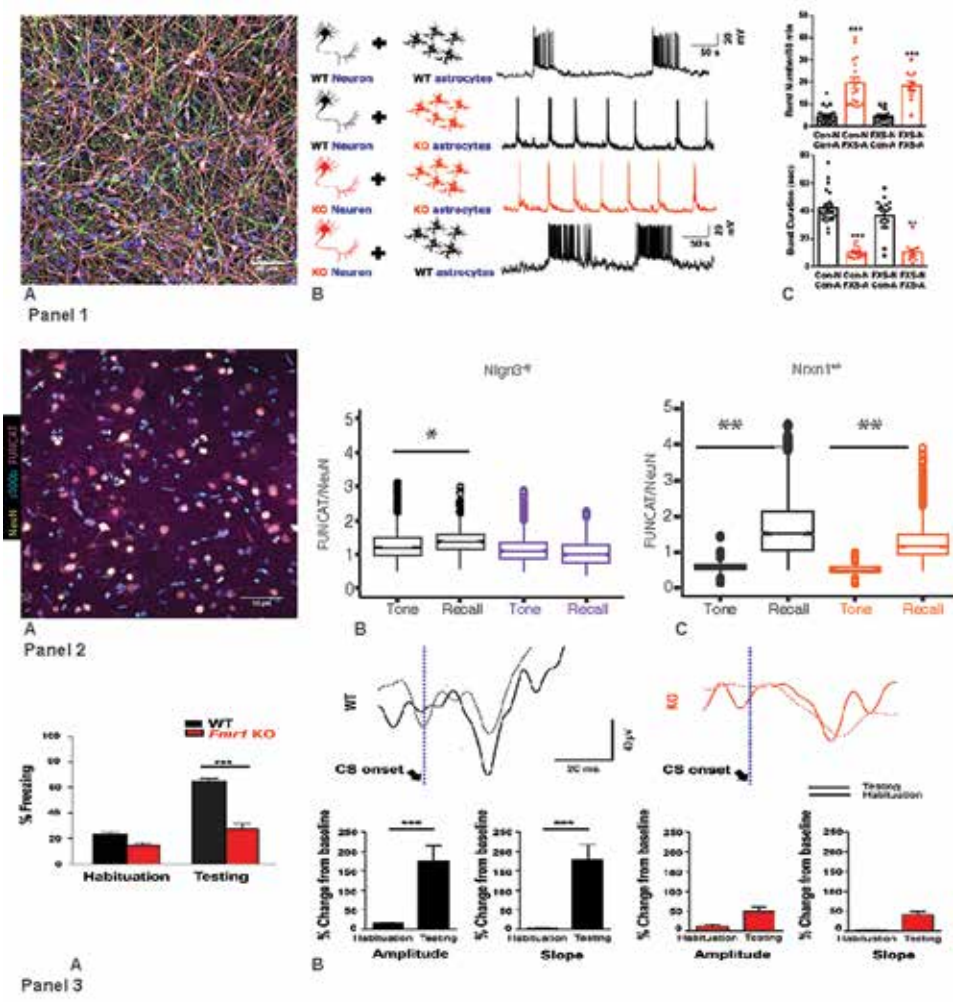
Programme 2: The autistic network – from pathways to rescue

Complex neurological diseases such as ASD are typified by altered behaviour in humans which can be modelled with some degree of precision in animals. Decades of research on behavioural alterations has established that such changes are usually wrought by massive changes in proteins, DNA methylation and gene activation. These molecular changes provide not only opportunities for bespoke therapeutics but also biomarker discovery for patient stratification in future clinical trials. While proteomic shifts have been well documented in syndromic ASD such as FXS, Tuberous Sclerosis and Syngap Haploinsufficiency, non-syndromic ASD have been less studied. It is also presently unclear how much of the proteome is restructured in neurons and glia.

We monitored tandem neuronal and glial protein synthesis in brains of Neuroligin 3 and Neurexin 1 rat models of non-syndromic ASD in response to a specific behavioural task. In the fear-recall task Neuroligin 3 animals showed impairments which correlated with impaired protein synthesis in neurons and astrocytes. Recall-induced freezing and proteomic shifts in Neurexin 1 model rats comparable to control rats. Finally, transactivating translation using specific molecules that can increase protein synthesis was able to rescue recall induced freezing. In summary, we have uncovered links of cell-specific protein synthesis in a given brain circuit to altered response in behavioural tasks. Simultaneously, non-syndromic ASD brains may also have altered proteomes, arguing for an underlying molecular convergence across disparate models of autism.

Programme 3: Autistic function – rat behaviour and imaging

In this study using a rat model of FXS we found that recall of fear memories formed during Pavlovian auditory fear conditioning is impaired in Fmr1-KO rats. To examine the neural basis of this deficit in the encoding and recall of fear memories formed by auditory fear conditioning, we carried out in vivo recordings of local field potentials (LFP) in the lateral nucleus of the amygdala (LA). This revealed that in the KO rats which showed impaired fear recall, both the slope and amplitude of LFPs evoked by the tone conditioned stimulus (CS) were reduced compared to their wild-type (WT) counterparts. Further, using whole-cell recordings in brain slices, we found a significant impairment in long-term potentiation (LTP) in LA principal neurons in KO rats.



Panel 1: *[A] Human iPSC derived neurons were co-cultured with the human iPSC derived astrocytes (β-III tubulin/ Map2ab/GFAP/DAPI- 20X) for 8 weeks in vitro to form a dense network of neurons and glia.* ***[B]*** *Glial modulation of the network activity of neurons and* ***[C]*** *Quantification of burst firing parameters – burst frequency calculated as number of bursts/10 min and burst duration.*

Panel 2: *[A] Localised cell-specific protein synthesis measured using FUNCAT (Fluorescent Non-canonical amino acid tagging. An amygdala brain slice stained with FUNCAT (red), NeuN (yellow) and S100b (blue) at 40x magnification.* ***[B and C]*** *Quantification of intensity of newly synthesised proteins in amygdalar brain slices of Neuroligin 3 -/Y (purple) and Neurexin 1 +/- slices (orange) relative to control (black) after a fear memory task. Impaired neuronal protein synthesis can be seen in Nlgn3 -/Y case.*

Panel 3: *[A] Fmr1-KO rats showed impairment in the recall of auditory fear memory: Fmr1-KO rats show a decrease in the freezing response after fear conditioning as compared to WT.* ***[B]*** *Auditory evoked potentials (AEPs) in response to the tone is lower in Fmr1-KO rats: WT rats exhibit a significant increase in the amplitude and slope of auditory evoked field potentials (AEPs), in response to the tone after fear conditioning. This increase in the tone induced AEPs is impaired in the amygdala of Fmr1-KO rats. Blue lines indicate the tone onset.*

7.2

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Protein Synthesis Defects in Human Stem Cell Models of Alzheimer's Disease

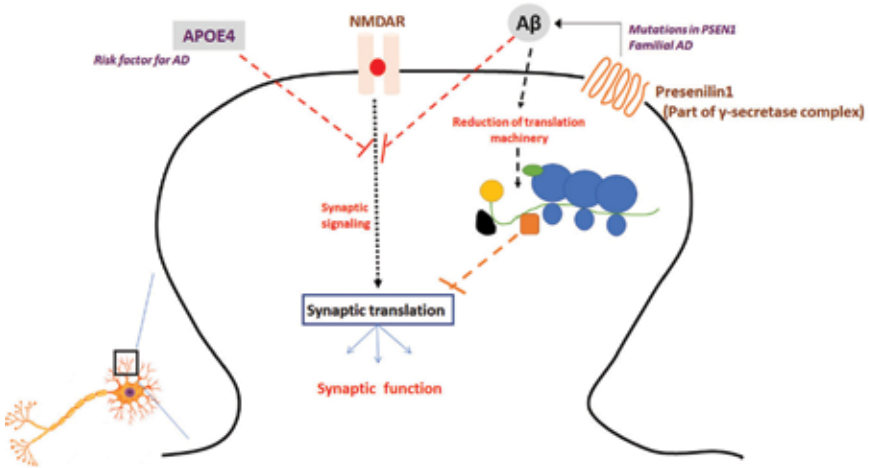
Using human stem models, we are able to show the defects in synaptic protein synthesis associated with Alzheimer's disease. Our work shows a deficiency in the translation machinery in neurons with PSEN mutations and defects in the key signalling components connecting glutamate receptors to translation machinery in the presence of ApoE4, a risk factor.

Understanding the molecular mechanism of synaptic protein synthesis and its contribution to synaptic plasticity is a core research interest for my group. In the past few years, we are able to elucidate the mechanism underlying the translation regulation of two important classes of glutamate receptors namely mGluR and NMDAR *(Kute P et al 2019, Dastidar SG et al 2019, and Muddashetty et al 2011)*. Our recent work shows that activity mediated protein synthesis plays a vital role during both neuronal development *(Ravindran S et al 2019)* and plasticity *(Kute P et al 2019).* Importantly, we also demonstrate a delicate balance between protein synthesis and energy metabolism at the synapse *(Dastidar SG et al 2019).* Since defects in energy metabolism and synaptic function are proposed to be central to the pathophysiology of Alzheimer's disease (AD), we hypothesised that dysregulation synaptic protein synthesis potentially has an important role in the synaptic defects observed in AD. To study this, we chose human stem cell-derived neurons from AD patients with the gene-corrected (GC) lines generated on the same genetic background using CRISPR-Cas9 technology.

In collaboration with the University of Copenhagen, we have characterised and optimised the methods to differentiate glutamatergic neurons from iPSC lines generated from AD patients (PSEN mutants) and their corresponding GC lines *(Frederiksen HR et al 2018)*. We studied both basal and activity mediated translation regulation using these cells. From this study, we made two interesting observations: **1)** There is a significant reduction in the translation machinery in mutant line compared to GC lines but surprisingly in this background, the synaptic mRNA level was significantly increased in the mutant lines. **2)** Synaptic activity mediated translation *(particularly NMDAR mediated)* was completely lost in neurons derived from mutant lines. These contrasting results indicate defects in protein synthesis at multiple levels in the stem cell-derived neurons of AD. Deficiency in translation machinery indicates a potential feed-forward inhibition due to the defective energy

metabolism in mutant lines and the disruption in the delicate balance between translation and energy dynamics. Deficiency in synaptic translation even with an over-abundance of synaptic mRNAs indicates the loss of signalling between glutamate receptors and translation machinery where increased Aβ peptide may have a direct role *(Figure).*

Apart from mutations *(like PSEN, APP, and other genes)* causing familial AD, there are many risk factors implicated both in familial and sporadic cases of AD. The ApoE4 allele is most potent among the risk factors studied so far but the biology through which it increases the risk is still not very clear. To study the effect of ApoE4 on synaptic translation we developed a novel system where we used the ApoE4 *(or other variants of ApoE)* secreted to the conditioned media from human iPSCs with the isogenic background *(Phren K et al 2018)* on cultured cortical neurons from Rat. This system allowed us to study the effect of different ApoE variants *(with ApoE knockout conditioned media as a control)* on the basal and activity mediated synaptic translation. Our results show that even an acute exposure *(20 minutes)* to ApoE4 significantly disrupts the basal translation which precludes the synaptic activity mediated (NMDAR) translation *(Figure)*. Our system is ideally suited to study the effect of ApoE4 and PSEN *(or APP)* mutants on synaptic translation individually or in combination. We are currently working on the molecular mechanism by which ApoE4 and Aβ affect synaptic translation and how it would contribute to the pathology of Alzheimer's disease.



Defects in synaptic protein synthesis in AD

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Research Talks

Sumantra Chattarji:

Uniquely Amygdala, Distinct patterns of plasticity caused by stress and autism - Dutch Neuroscience Meeting, Keynote Lecture, Lunteren, Netherlands, 2019.

Enriched environment and stress resilience: Make hippocampus great again! - Spring Hippocampal Research Conference, Invited talk, Taormina, Sicily, Italy, 2019.

Fragile X Syndrome and Fear: Alternative facts from the amygdala - Proteins and Circuits in Memory, Invited talk, Copenhagen, Denmark, 2019.

Fear and FXS: Alternative facts from the amygdala. From synapses to memory: RNA based regulatory mechanisms - India-EMBO Symposium, Invited talk, Denmark, 2019.

Fear and FXS: Alternative facts from the amygdala - Gordon Research Conference, Invited talk, Fragile X and Autism-Related Disorders, Italy, 2018.

mGluR-dependent plasticity in the amygdala in a rat model of FXS - Simons Initiative for the Developing Brain, Annual Scientific Review, University of Edinburgh, UK, 2018.

Fighting fire with fire - Stress-Neurobiology Workshop, Invited talk, Banff, Canada, 2018.

FXS, mGluR-dependent plasticity and the amygdala - Autism Research Initiative, Science Meeting, Aarhus University, Denmark, 2018.

Stress, emotional memories and the amygdala - PROMEMO-Mini-symposium on Memory, Inaugural Plenary Lecture Aarhus University, Denmark, 2018.

Ravi Muddashetty:

FMRP and specialised Ribosomes - EMBO meeting-NBRC, Manesar, October 2018.

"Pushes and Pulls" of synaptic translation - Centre for Neuroscience, IISc, Bangalore, India July 2019.

Outreach

Sumantra Chattarji:

Popular Science Articles

A bipolar route to updating old memories - https://indiabioscience.org/news/2019/a-bipolar-route- to-updating-old-memories, July 2019.

How depression affects memory? - https://neurosciencenews.com/stress-neuroimaging-memory-hippocampus- 4735/ , April 2019.

Stress elicits contrasting effects on the structure and number of astrocytes in the amygdala versus hippocampus - https://www.eneuro.org/content/6/1/ENEURO.0338-18.2019, January 2019.

Some bad memories can be forgotten - https://www.techexplorist.com/experiments-rats-show-bad-memories-forgotten/17164/ , September 2018

Nutrients of neuroscience: Learning from animal studies could make our children flourish and enhance memory -https://www.theweek.in/health/more/2018/08/31/nutrients-of-neuroscience.html, September 2018.

Ravi Muddashetty:

Memory and Synapse exhibition - Science Day, inStem-NCBS campus, November 2018.

Memory and synapse exhibition - Swamy Vivekananda high school, Saraguru, Mysore District, December 2018.

Lab visit by school children, July 2019.

How do we learn and why do we forget - Science café, Bangalore, July 2018.

Neurobiology of Memory - workshop for Biology teachers, Kuvempu University, Shivamoga, November 2018

7.3

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ADBS: Accelerator Programme for Discovery in Brain Disorders Using Stem Cells

The ADBS is a venture to understand the genetic and cellular basis of severe mental illness by harnessing the power of modern human genetics and stem cell technology. The programme is a collaborative initiative of three institutions from Bengaluru, India – the Institute for Stem Cell Science and Regenerative Medicine (inStem), the National Centre for Biological Sciences (NCBS) and the National Institute for Mental Health and Neurosciences (NIMHANS). This programme uses modern stem cell technology to create cellular models of the brain derived from human subjects with a strong history of severe mental illness. The overall goal is to uncover the genetic, cellular and molecular basis of mental illness and relate these to clinical findings.

Severe mental illness 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 this huge disease burden, the development of novel ways to diagnose and treat mental illness will have important positive social and economic benefits. To achieve this goal, there is a pressing need to understand the mechanistic basis of these disorders; such discovery could form the basis for the development of novel diagnostic and therapeutic approaches.

The ADBS programme studies five major forms of severe mental illness (SMI): schizophrenia, bipolar disorder, obsessive compulsive disorder, substance dependence and dementia. All of these disorders are known to have an inherited basis. However, despite their high heritability, to date few genetic correlates that could account for of this high heritability have been identified. In order to study these disorders, in collaboration with the Department of Psychiatry, NIMHANS and NCBS, inStem has assembled a prospective cohort of families with a strong family history of SMI. The ADBS programme is pursuing three distinct but interactive lines of analysis on these families: **(i)** The families have been clinically studied in depth to understand changes in structure and function at multiple levels of brain organisation; they will now be followed over a period of twenty years at 3 year intervals in order to define the temporal development of disease through regular and detailed clinical phenotyping. **(ii)** We have established induced pluripotent stem cell

lines from affected individuals in these families and unaffected controls. These lines are being used to generate cellular models and mechanistic aspects of cellular neurobiology that lead to disease. **(iii)** Next Generation Sequencing and family-based bioinformatics analysis is being used to uncover the genetic basis of SMI.

The multiple types of data generated by the ADBS programme are being assembled into an integrated database to facilitate the application of sophisticated methods of data analysis to uncover new disease biology. The stem cell lines and other biomaterials have been assembled into a biorepository that will allow the sharing and use of this resource to drive discovery biology in the area of SMI. The ADBS programme has instituted mechanisms to facilitate the sharing of data and resources generated through its activities.

A key objective of the ADBS programme is to expand and facilitate the application of modern stem cell technology and genomics for discovery in human disease biology in India. To this end, ADBS has undertaken a series of initiatives:

Research Training programmes for post-graduate level researchers

(i) CiRA-ADBS training programme in human induced pluripotent stem cell technology: The CiRA-ADBS training programme on generation and maintenance of human induced pluripotent stem (iPS) cells is organised annually by the Accelerating the application of Stem cell technology in Human Diseases (ASHD) programme and the Centre for iPS Cell Research and Application (CiRA), Kyoto University, Japan. The goal is to expand the expertise for state-of-the-art iPSC based research within India. As part of this effort, every year CiRA hosts Indian researchers at its laboratories in Kyoto, Japan for a training programme that features instruction on methods for working with human iPS cells. Twenty one Indian researchers have been trained through this programme over the past three years. Eight Indian researchers *(two postdoctoral fellows, six postgraduates)* from across India attended this training programme during November 2018.

(ii) In collaboration with the Institute of Bioinformatics and Applied Biotechnology (IBAB), Bengaluru, the ADBS programme organised a three day hands-on training programme on Whole Genome Sequence Analysis in December, 2018. The training programme was designed to train individuals on R programming, whole genome sequence assembly, Haplotype calling, INDEL and SNP calling, SNP extraction and INDEL extraction. The workshop included both lectures and practical sessions. Eighteen young researchers *(sixteen post-graduates and two postdoctoral fellows)* from institutions across India attended the workshop.

(iii) The second ADBS Human iPSC Workshop on *"Reprogramming Human Somatic Cells to HiPSCs"* was held during June 3rd-14th, at inStem, Bangalore. Twelve researchers from across India attended this hands-on training programme. The workshop included lectures by leading experts in the field and practical sessions on cell culture techniques involved in generation, characterisation, propagation and differentiation of HiPSCs.

Scientific meetings organised by ADBS

Human genetics and disease biology meeting: The sequencing of the human genome has raised much excitement in the possibility of understanding the blueprint of life. Further, the development of low-cost high-throughput DNA sequencing has facilitated the analysis of large numbers of human genomes and revealed the diversity in DNA sequence

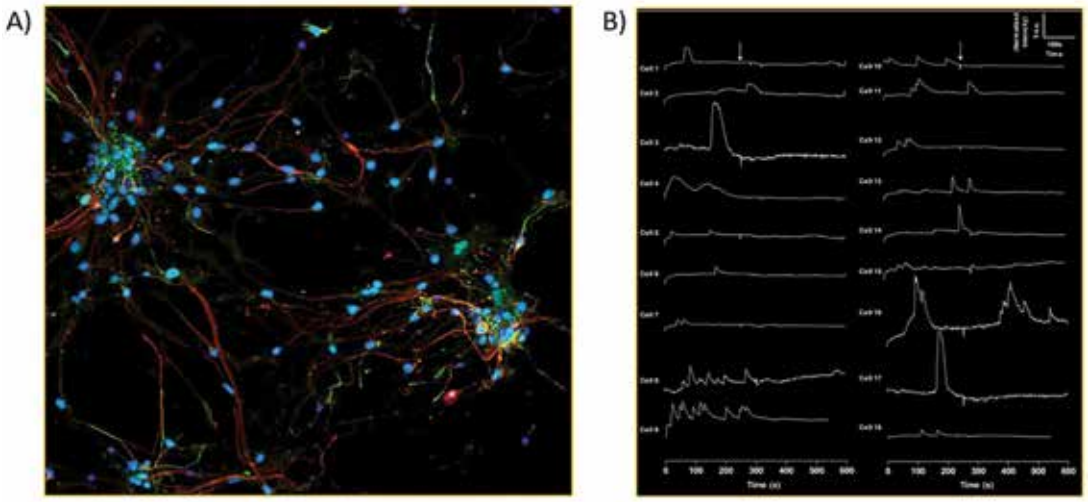
within and between human populations. Modern human genomics has also raised the possibility of identifying DNA sequence changes that may be linked to diseases such as diabetes, cancer and mental illness as well as facilitated new approaches to understanding issues such as human migration, evolution and population structure. Upholding the importance of genomics research in human disease biology, the ADBS programme coordinated the Human Genetics and Disease Biology Meeting on 8th June, 2018. This meeting brought together eminent leaders in academia and industry to discuss the opportunities and challenges for studies of human genetics and disease biology in India using new advances in DNA sequencing technology.

Outreach

Prof. Raghu Padinjat discussed mental illness and *'Applying modern technologies to understand diseases of the brain'* at Science café on May 26th, 2019, at Suruchi Rangamane, Mysore.

Full details of the ADBS programme can be found at the following webpage: *https://www.ncbs.res.in/adbs/*

Ongoing work and progress of the ADBS programme are also communicated via our Twitter handle: *@BrainStem_ADBS* and the Facebook page of the programme: *https://tinyurl.com/y2cjerye*



Biochemical and functional characterisation of human NSC-derived neurons: (A) hNSC-derived neurons express doublecortin (DCX, green) and beta-3 tubulin (red), and nucleus stained with DAPI. (B) 30 days old neurons exhibit spontaneous activity detected using calcium imaging.

Publications

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Research Talks

Raghu Padinjat:

Modelling neurodevelopmental disorders using stem cells - Pediatric Research and Education Society of India, 3rd Annual Conference, Bangalore, India, November 2018.

Defining cellular mechanisms to derive personalised solutions for mental illness - 7th International Symposium on Current Trends in Drug Discovery Research, Central Drug Research Institute, Lucknow, India, December 2018.

Multiscale modelling of human physiology in health and disease - 87th Annual Conference of the Society of Biological Chemists of India, Manipal Academy of Higher Education, Karnataka, India, December 2018.

44th annual meeting of the indian society for human genetics - NIBMG, Kalyani, West Bengal, India, February 2019.

School of regenerative medicine - Manipal Academy of Higher Education, Bangalore, India, February 2019.

Connaisance - 3rd International Undergraduate Medical Conference, JIPMER Pondicherry, India, April 2019.

Advancements in biological sciences and its applications - Karnataka Science and Technology Academy, Bangalore, India, March 2019.

8

CCBD

Centre for Cardiovascular Biology and Disease

The Center for Cardiovascular Biology and Disease theme in inStem focuses on genetic hypertrophic and dilated cardiomyopathies, autosomal dominant myocardial diseases caused by missense mutations in any one of the genes encoding the fundamental contractile apparatus of the heart. These diseases are common and are debilitating and often lead to sudden death. 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 the 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.

Theme Coordinators:

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8.1

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Structure and Function of Cytoskeleton and Motility Systems

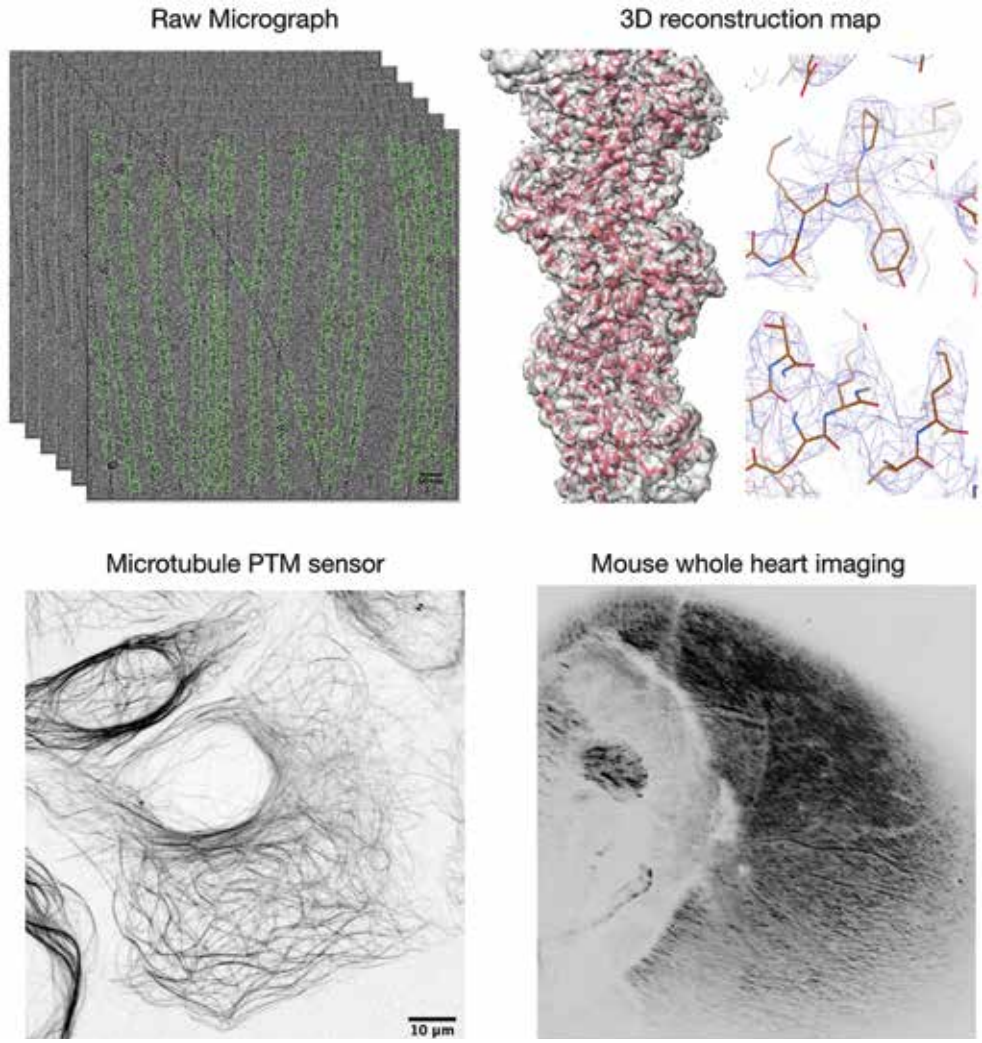
Eukaryotic biological motions across orders of magnitude scale involve cytoskeleton elements. Mutations in them are frequently associated with human pathology, e.g., cardiomyopathies, neurological disorders and ciliopathies. Our lab studies the cytoskeleton systems using CryoEM, biochemistry, cell biology and microscopy, thereby bridging the knowledge gap between clinical findings and molecular mechanism.

As a part of cardiomyopathy team, our lab research focuses on bridging the knowledge gap between clinical findings and molecular mechanism underlying cardiomyopathy disease causing mutations. Currently our group has setup pipeline for Cryo-Electron Microscopy (CryoEM) high-resolution structure determination, biochemistry, cell biology and microscopy methods to study cardiomyopathy causing mutations and their disease mechanism. Together our theme covers human genetics, animal models, cell biology, biochemistry, and structure at the molecular level of cardiomyopathy diseases.

In order to address the molecular changes i.e., what biochemical and structural changes occur upon cardiomyopathy causing mutations, we have chosen nexilin as a case study . Nexilin is an actin binding protein present in Z-discs of the sarcomere. Clinical studies have identified 5 mutations and the mutations that cause HCM and DCM are clustered at the amino- and carboxy-terminal of the nexilin respectively. In addition, Perundurai lab has already identified a novel mutation in nexilin that is present in the Indian population. However, there are no reports of structural or functional characterisation so far. Our work in this direction includes purification of full-length, truncations and mutant nexilin proteins, actin binding assays and CryoEM of nexilin bound to filamentous actin *(Figure)*. Future work will involve introducing this mutation in mice to study and model the human patient mutation effects.

At the cellular level, our lab is working towards understanding the microtubule cytoskeleton in particular tubulin posttranslational modification (PTM) and organisation *(Rostovtseva et al., 2018)*. Recent reports suggest detyrosinated tubulin (tubulin PTM) levels are elevated during cardiomyopathies. In this regard, we have generated a live cell sensor to detect these PTMs *(Figure)*. These sensors will be valuable in understanding how microtubule growth, dynamics and stability is governed by tubulin PTMs and their dysregulation during cardiomyopathies. We have an active ongoing collaboration with Dr. Carsten Janke from Curie Institute, France to tackle tubulin PTMs, which is funded by a joint CEFIPRA grant between our labs.

In our quest to understand cardiomyopathy, we have established methods to determine the muscle fiber organisation of heart walls at cellular resolution. Cardiomyopathies have been frequently associated with fibrosis and sarcomere disarray, in order to find out at which scale the fibers are disarrayed we will employ microscopy methods to compare wildtype and diseased hearts *(Figure).* These methods will be invaluable to address the heart morphological changes during cardiomyopathies.



Cryo Electron Microscopy micrographs of F-actin, green circles represent the extraction of particles for averaging (top, right). Three-dimensional map of F-actin filament (top, middle), closer view of amino acids fitted in electron map of F-actin structure (top, left). Microtubules in tissue culture cells labeled with live cell sensor against tyrosinated state of tubulin PTM (below, right). Whole organ image of cadavered mouse heart using macro confocal microscope (below, left).

8.2

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Functional Genomics of Cardiomyopathies

Cardiomyopathies are a group of heart muscle diseases that often lead to progressive heart failure with significant mortality. The cause of a significant percentage of cardiomyopathies (~40%) remains unknown with poorly defined mechanisms and no curative therapies. To address these questions, our group encompasses a multi-disciplinary approach involving Next Generation Sequencing (NGS) in identifying new genes and various models to understand the mechanistic basis and therapeutic targets for the new cardiomyopathy genes.

Whole exome sequencing to identify novel genes for cardiomyopathies

We have organised fifty unrelated Indian cardiomyopathy patients *(who are negative for reported genes)* and their family members *(a total of 150 individuals)*. We performed whole-exome sequencing in selected index patients with their respective family members as controls. In two unrelated patients, we identified two novel mutations in Adiponectin Receptor R1. We are exploring the mechanisms of these genes using cellular models. For the remaining patient samples, the exome analysis is in progress.

Patient-specific induced pluripotent stem cells (iPSC)-derived cardiomyocytes

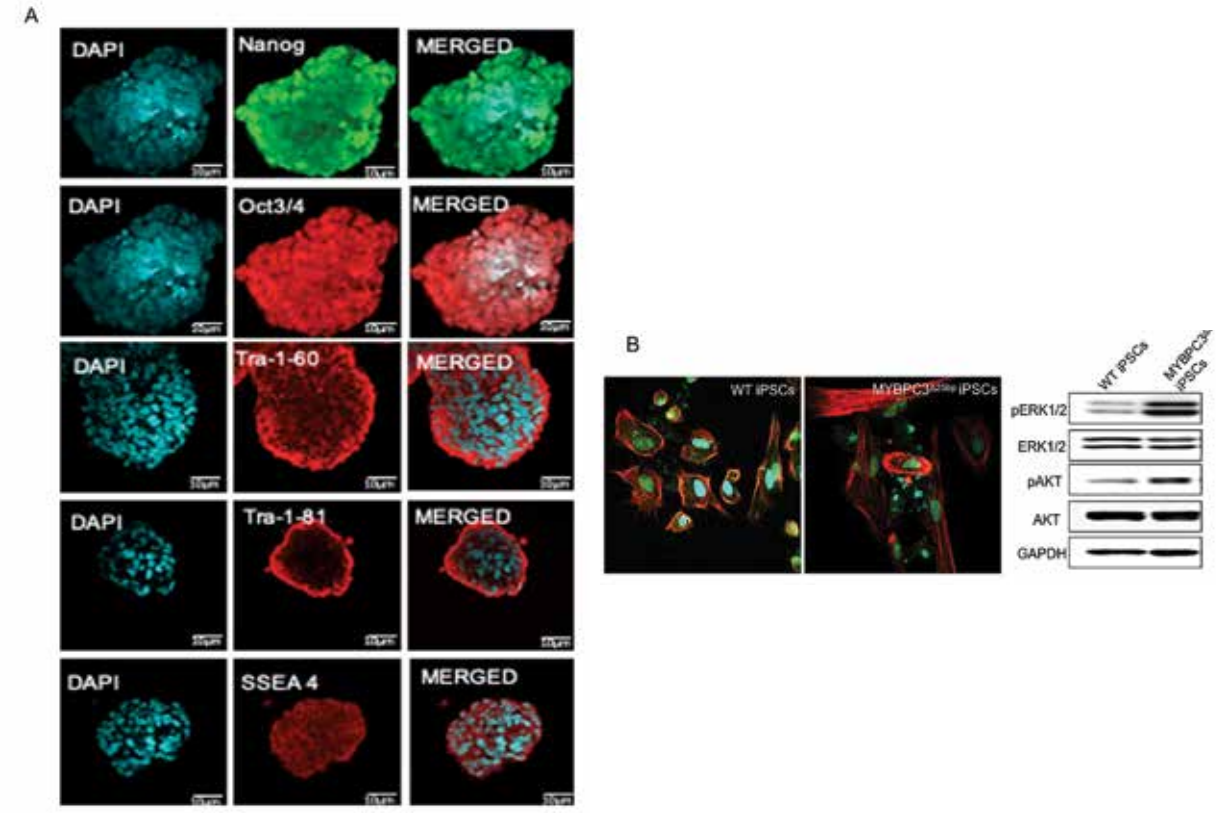
Our research work on the molecular genetics of cardiomyopathies has identified an ancient common variant (25bp deletion) associated with cardiomyopathies in cardiac myosin-binding protein C3 (MYBPC3) gene in South Asians. This variant, in its homozygous nature, causes severe childhood cardiomyopathies. We have generated and purified the cardiomyocytes from patient iPSCs harboring the MYBPC3 variant respectively *(Figure)*. This Indian patient-specific iPSCs line will be helpful in understanding the molecular mechanisms and identifying new candidate therapeutics for cardiomyopathy.

Humanised transgenic mice models of cardiomyopathies

We have generated a humanised cardiac-specific transgenic mouse model for MYBPC3 (25bp variant) using standard Cre-loxP recombination methods. We are characterising the physiological, functional, and molecular aspects of the MYBPC3 transgenic mouse model. In addition, we have generated a transgenic mouse model with a novel PRKCA mutation observed in children cardiomyopathies. The PRKCA mutant mice develop cardiomyopathy at around four weeks after birth. The histological analyses in these mouse hearts revealed massive cardiomyopathy with the hallmarks of hypertrophy, including increased cells sizes and myocardial fibrosis. Both these mice models will help in understanding the disease pathogenesis and preclinical drug validation.

Cardio-protective genes in Indians

According to a report from the World Health Organisation *(https://www.who.int/gho/publications/world_health_statistics/2016/en),* the average life expectancy at birth of the global population is 72 years, and in India, it is only 68 years. Currently, available disease prevention methods and a healthy lifestyle have helped in increased lifespan or longevity. Longevity and healthy aging are closely related; healthy aging is defined as human aged 80 years or more with the ability to enjoy healthy conditions both mentally and physically in the absence of chronic illness and medical interventions. It is well-known that few genetic signatures have been strongly associated with longevity and healthy aging. However, most of these genetic association studies are done in western populations. Our aim for this study is to sequence as many as healthy aging individuals from India *(at least 2000 healthy aging individuals)*. At present, we have organised 203 samples. Meanwhile, as a proof of principle, we have compiled 94 exomes and successfully established the database *(www.idhans.org).* We identified various Indian specific cardioprotective genes. This data will help the scientists and clinicians across the globe for analysing various clinically-associated and protective variants.



Representative image of generation of pluripotent stem cells (A), cardiomyocytes differentiation and signalling pathway analysis (B) from an HCM patient with a MYBPC3 mutant

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Research Talks

Minhaj Sirajuddin:

Research Lecture at Bangalore Microscopy Course, Bangalore, India, September 2018.

Research Talk at British Microtubule Meeting, Edinburgh, UK, May 2019.

Guest Lecture at Wellcome Trust Center for Cell Biology, Edinburgh, UK, May 2019.

Guest Lecture at University of Oxford, Oxford, UK, May 2019.

Guest Lecture at Warwick University, Warwick, UK, May 2019.

Dhandapany Perundurai:

Functional genomics of cardiomyopathy - Invited speaker and Chairperson for the cardiomyopathy section in the World Congress on Cardiac Sciences 2018 organised by BioGenesis Health Cluster, UK and held from November 28–29th, 2018 at J N Tata Auditorium, Bangalore, Karnataka, India.

Genetic etiology of hypertrophic cardiomyopathy - Invited speaker for American Heart Association to be held on November 16-18th, 2019, Philadelphia, USA.

Outreach

Minhaj Sirajuddin:

We created a **YouTube video** describing how molecular motors are involved in color change in animals, with additional information for DIY style practicum instructions.

Video title: **Color change with fish scale melanocytes and DIY instructions**

Video link: **https://www.youtube.com/watch?v=hyr2lCFF260**

9
TAS

Technologies for the Advancement of Science

The work in TAS theme is about understanding life processes from the molecular to organismal level and at different time scales. Our variety is our strength and our work is primarily driven by collaborations within the group, the institute and the wider scientific community. For instance, using regenerative and stem cell models, we made fundamental discoveries in natural light sensing and translation regulation that have been widely recognised. We developed biosensors and probes for bioimaging of proteins and organelles, which provided insights into the mechanism that regulate cell behaviour. We also developed new materials and solved structures that have significant impact on biomedical research such as alleviation of inflammation and tackling the problem of biofilm formation. Overall, our research achievements driven by collaborative vision lead to the establishment of molecular and chemical tools to study difficult problems in fundamental and applied biology.

Theme Coordinator:
S Ramaswamy

9.1

S
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The Molecular Form and Function Lab

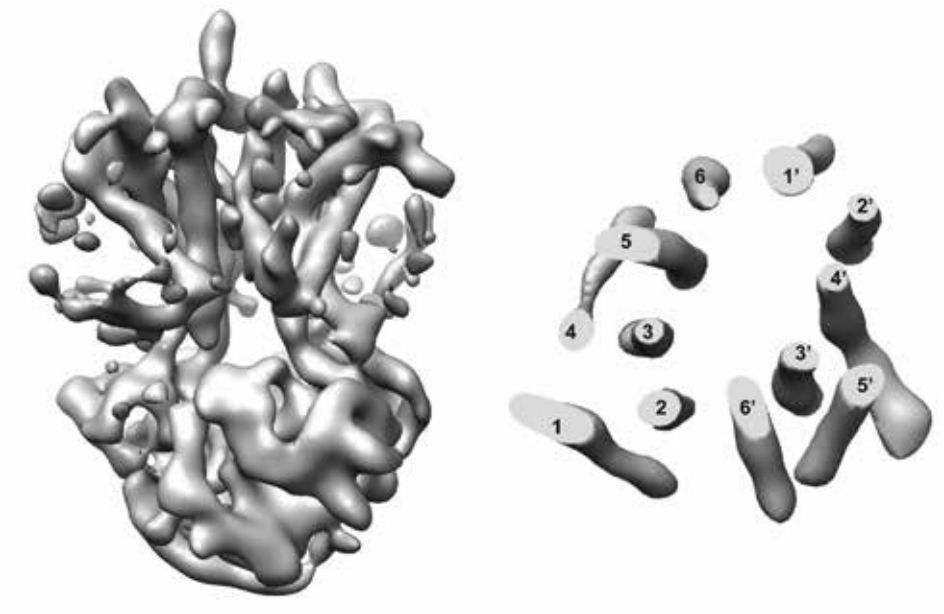
The primary interest of the laboratory is to understand biology from the first principles of physics and chemistry. The detailed molecular insights allow for the manipulation of biology for applications in human health and welfare. Over the last year, the main focus has been on understanding nine carbon sugar metabolisms at the molecular level.

The lab's interest in sugar transport and metabolism has led to more publications this year as many of the students begin to finish their PhD and post-docs finish their fellowships. Jayprakash Kumar completed his PhD and has moved on to a post-doctoral position at the NIH in Bethesda. Lavanyaa Manjunath has finished her PhD and after a break has moved to a post-doctoral position at York in the Scotland. Sanchari, a post-doctoral fellow, who did a lot of work on the cockroach milk protein has found a position as a PI at the Institute of Bioinformatics and Applied Biotechnology – IBAB, Bangalore.

Several commensal gram-negative bacteria, when immunocompromised, often steal host Neu5Ac and display it as the outermost sugar to avoid immune response from the host. This results in the bacteria multiplying freely and often causing sepsis. Several bacteria also routinely use Neu5Ac in the gut or in the oral cavity as a carbon source. Our laboratory has worked for several years now on the catabolic pathway of how Neu5Ac when scavenged by gram-negative bacteria is used as a carbon source. We have determined structures of all the proteins in this pathway. Once the Neu5Ac is transported into the cytoplasm the other pathway is the ability of these bacteria to coat themselves with Neu5Ac. The first step in this is the addition of a nucleotide to the sugar by the enzyme CMP-Neu5Ac synthetase. The second step is the enzyme Sialyl transferase. This enzyme adds the Neu5Ac as the outermost sugar of the glycolipid. This is a membrane anchored enzyme. Structures of just the soluble domain of the enzyme have been determined. Using electron cryo-Microscopy we have now been able to get initial maps of this enzyme with the membrane helix in detergent micelles.

The import of the sialic acids into the bacteria from the periplasm to the cytoplasm can happen, either through a TRAP transporter, an ABC type transporter or an SSS type transporter. In the last year, we published the structure of a SSS-type membrane transporter. We have been working on understanding at the molecular level how the ABC and the TRAP transporters function. We have made interesting progress in this regard as well. In collaboration with Vinothkumar at NCBS, Parveen Goyal, a post-doc in the lab, has preliminary cryo-EM maps of an ATP dependent sialic acid transporter from H. ducreyi *(Figure).* In the coming months, we hope to have the high-resolution structure of this protein as well, that will allow us to then ask specific molecular determinants that provide specificity of Neu5Ac transport to this large class of ABC transporters.

Interestingly, two different IPs from the lab are in the final stages of technology transfer. Our work on how yeast makes insect inspired high calorie protein *(the infamous cockroach milk)* is one of them. The other is our work on purifying venom from bees that have significant healing properties in arthritis. We hope these will be completed soon and we will have products in the market from knowledge generated in the laboratory at inStem.



Cryo-EM map of sialic acid ABC transporter of H. ducreyi: The ABC transporter was purified in detergent to be used for cryo-EM. Data was collected using Titak Krios 300KV in counting mode. 120,000 particles were picked from 1120 micrographs and used for 2D classification. 3D model was built and refined up to 6.5Å. At this resolution, arrangement of the α-helices was clearly visible. We were able to distinguish between transmembrane domain and ATPase domain.

9.2

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RNA Mediated Regulation of Stem Cell Function and Regeneration

Identifying factors that regulate protein synthesis is essential to understand the mechanisms that alter the protein repertoire critical for stem cell state transition. Using mammalian embryonic stem cells, and the regenerative model Planaria, Schmidtea mediterranea, we identified several cis and trans regulators that interact with ribosome and regulate the efficiency of translation important for stem cell function.

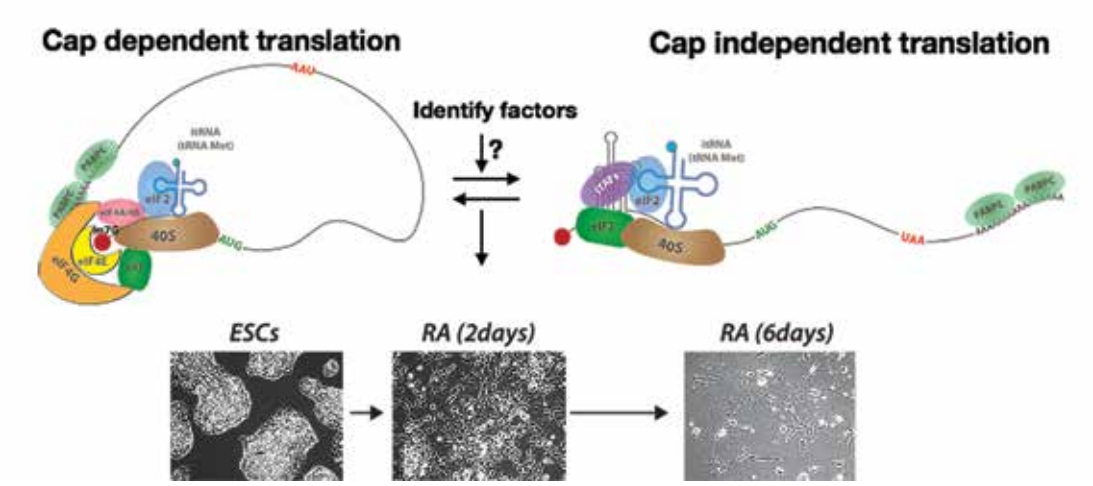
Ribosome centric regulation of translation critical for stem cell function

Ribosomes are the key component of translation machinery, which primarily consists of rRNA and ribosomal proteins. It has been assumed that ribosomes are monolithic structures that load on to the messenger RNA and help decode the information to generate cognate proteins. However, recent studies have shown that the changes in the composition of the ribosomes either by post-transcriptional modification of rRNA or post-translational modification of ribosomal proteins could alter the translation state, thereby changing the protein repertoire in the cell. My laboratory studies the role of both rRNA and ribosomal proteins in regulating protein synthesis critical for stem cell function.

Expansion segments of rRNA and their role in translation regulation: Computational analysis identified certain regions in the rRNA that were specifically evolved in humans and these regions are termed as expansion segments. Further analysis revealed that one of the expansion segments showed 10-13nt complementarity with the specific transcripts in the 5' UTR regions near the start codon. These transcripts were mostly transcription factors involved in developmental regulation. Pull down of this expansion segment identified various RNA binding proteins involved in translation regulation. Currently, we are testing the hypothesis that this expansion segment is important for loading of ribosome at the start codon and the interaction of this segment with RNA binding proteins is critical for the initiation of translation elongation.

Post-translational modification of ribosomal proteins mediate translation state changes during stem cell differentiation: Translation regulation is majorly regulated by the binding of the RNA binding proteins or various classes of small RNAs to the ribosomes. The binding of this proteins is mediated by post-translational modification of the ribosomal proteins. Of all the post-translational modifications known, the most reported is the phosphorylation of ribosomal protein small subunit-6 (RPS6). The RPS6 phosphorylation was mostly used as a marker for mTOR signalling and cap-dependent translation. However, the functional role of RPS6 phosphorylation in regulating translation is not

known. We found dynamic changes in the activation of components in mTOR signalling as well as cap dependent translation and RPS6 phosphorylation during initial time-points of stem cell differentiation upon LIF *(Leukaemia Inhibitory Factor)* withdrawal. Here, we hypothesise that translation state changes lead to alteration in the protein repertoire critical for stem cell proliferation and differentiation. Currently, we are performing extensive biochemical studies followed by the knockdown of the components of cap-dependent translation regulators to study their role in stem cell function.



Schematic showing the change in translation states critical for stem cell function. Our study aims at identifying factors that are essential for the change in mode of translation.

9.3

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Laboratory of Self-Assembled Biomaterials and Chemical Biology

The primary focus of the lab is to develop a wide range of biomaterials, bioengineering and chemical biology concepts to solve unmet clinical needs. Our lab discovered that the gut microbial metabolite, Urolithin A, and its novel synthetic analogues, offer dramatic protection against inflammatory bowel diseases (IBDs) by enhancing gut barrier function.

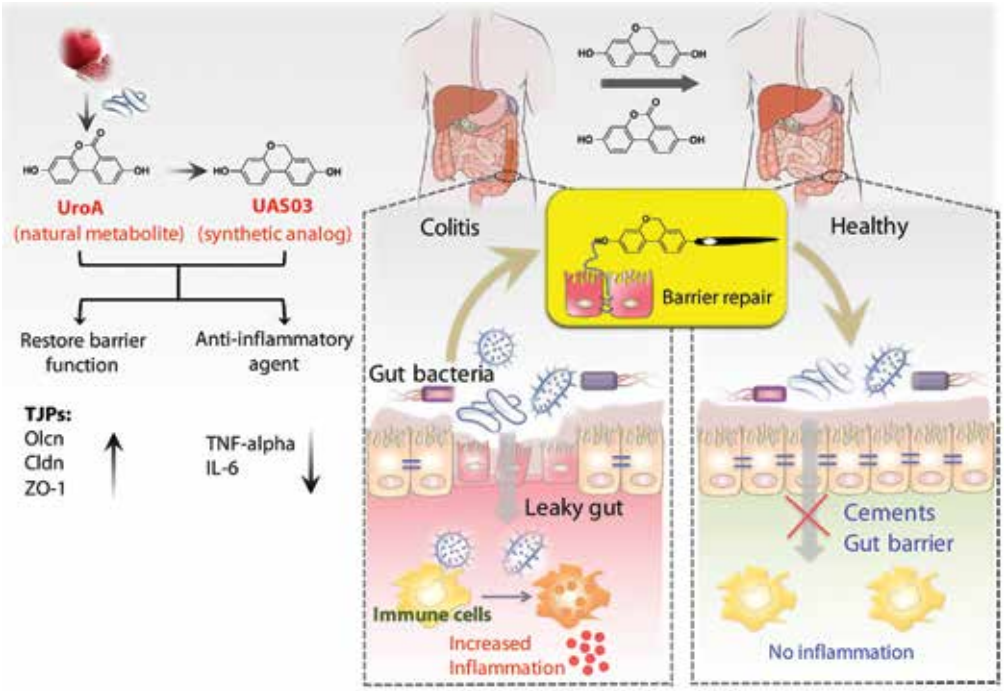
IBD consisting of Crohn's and Ulcerative Colitis are a result of the dysregulation of the immune system leading to intestinal inflammation and microbial dysbiosis. Gut barrier integrity is maintained by the tight junction proteins such as claudins (Cldn), Zona occludin-1 (ZO1), and occludin (Ocln) that are critical for epithelial cell barrier functions. It is known that levels of tight junction proteins are significantly down regulated under IBD conditions leading to increased gut permeability to microbial ligands and noxious metabolites resulting in systemic inflammatory responses. However, thus far, all treatment methods are using anti-inflammatory agents and immunosuppressants. Therefore, the current drugs can only manage the disease but do not cure. We hypothesised that restoring the gut barrier function by repairing the damaged epithelial layer by inducing the tight junction proteins (TJPs) will have enhanced efficacy in the treatment of IBDs.

Microbiota and their metabolites are in close proximity to the gut epithelium that constitutes a single cell-layer separating host components from the external environment. Consumption of diets containing berries and pomegranates have demonstrated significant beneficial effects on human health. It has been suggested that further downstream metabolites of ellagic acid (EA) known as 'urolithins' generated by gut microbiota render potential health benefits, in vivo. Among urolithins, Urolithin A (UroA) displayed potent anti-inflammatory, anti-oxidative and anti-ageing properties compared to other metabolites. Thus, not only the consumption of diets enriched in polyphenols is required but also the presence of microbes that convert them into beneficial metabolites is critical for manifestation of their health effects. At this time, the targets or pathways through which such microbial metabolites regulate physiological processes are largely unknown. Therefore, we have systematically investigated the role of UroA in providing beneficial effects and identified a novel mechanism in which UroA exhibits its efficacy. Briefly, we examined the activities of UroA identified that in addition to the anti-inflammatory activities, this compound strongly enhanced the gut barrier function. We demonstrated that UroA enhances barrier function by inducing tight junction proteins through activating Aryl hydrocarbon Receptor (AhR)-nuclear factor erythroid 2–related factor 2 (Nrf2)-dependent pathways.

UroA has a lactone that connects two mono-hydroxyl phenyl rings leading to a planar structure. Gastric pH or digestive enzymes can hydrolyse the lactone ring, which opens the ring resulting in the loss of the planar structure and potentially its activities. To generate more stable and potent compounds, we synthesised non-hydrolysable cyclic ether derivative, UAS03. The stability studies showed that UAS03 indeed is stable at gastric pH and also in the presence of gastric enzymes e.g., esterases and proteases.

Anti-inflammatory activities of UroA and UAS03 were examined in vivo in a LPS-induced peritonitis mouse model. UroA or UAS03 treatment significantly reduced the LPS-induced increase in serum IL-6 and TNF-α levels. Our results suggest that UAS03 is a potent structural analogue of UroA with increased anti-inflammatory activities. Our studies highlight the critical requirement for AhR-Nrf2 in protecting from barrier dysfunction. It is possible that UroA/UAS03 are exerting colitis protective activities by two pronged mechanisms of action. These compounds directly act on immune cells *(e.g., macrophages)* to prevent LPS/bacterial induced inflammation as well as exhibit anti-oxidative activities through AhR-Nrf2 pathways. Most importantly, these metabolites have direct impact on gut epithelium and gut barrier function by upregulating tight junction proteins. Enhanced barrier function reduces the bacterial leakage in the gut leading to significant reduction in systemic inflammation.

Overall, UroA/UAS03 will not only be efficacious in IBD-related diseases but may also have significant translational implications in other disorders involving barrier dysfunction and inflammation such as alcohol liver diseases, neurological disorders, and colon cancers.



A schematic picture of protection of gut barrier function using urolithins. Gut microbes produce metabolite Urolithin A (UroA) from pomegranate rich-diet, and a synthetic analog has been developed. Both molecules enhances barrier function through overexpression of tight junction proteins, and they act as anti-inflammatory agents.

9.4

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Engineered and Natural Biological Sensors

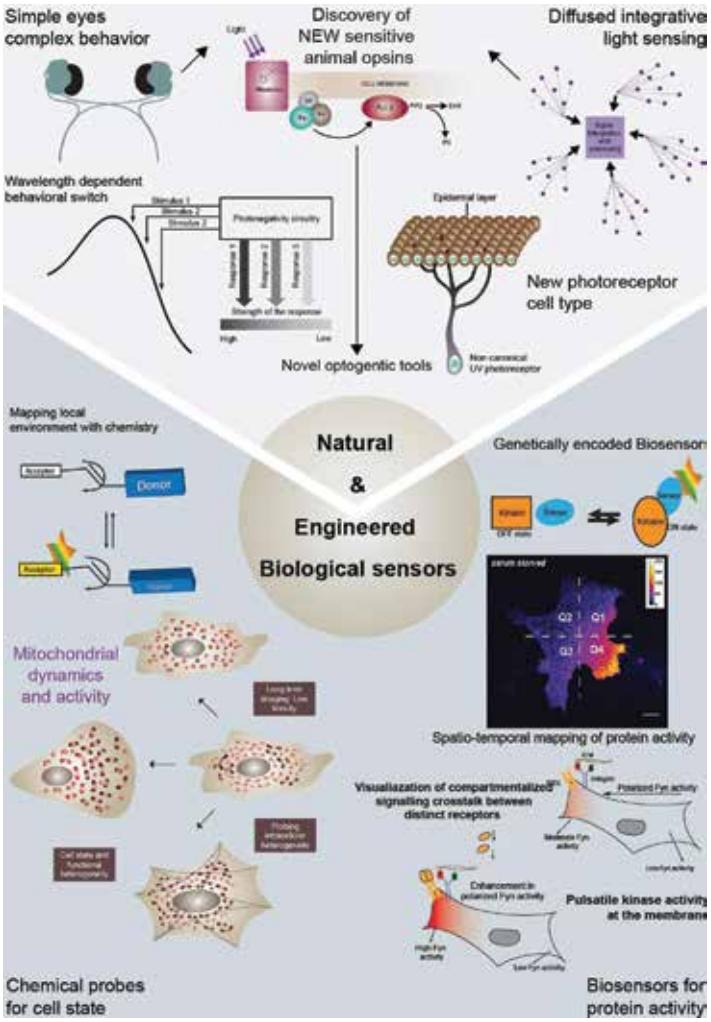
Our lab develops new biosensors and probes for visualising cellular dynamics. These fluorescent biosensors not only provide fundamental insights but are also useful for cellular diagnostics. We have discovered new light-sensing modules in nature paving way for new technologies for use in optogenetics and biomimetic light-sensing devices for human health.

Cells and tissues are constantly in flux. Cellular processes that control both regular cell function and disease pathologies rapidly change over time. Therefore, methods that enable probing cellular biochemistry in real time are extremely valuable. We have developed a range of engineered biological sensors and probes that allow visualisation of cellular dynamics with spatio-temporal precision. In cells protein activity is tightly regulated and often the precise activation of signalling proteins control cell fate and disease pathology. We have developed biosensors to directly image active conformations of signalling proteins. A new biosensor for a critical cellular kinase Fyn belonging to the Src family has shown how kinase activity is tightly controlled in space and time. Protein activity does not remain static but shows temporal oscillations. We also find that activity patterns are conserved in different cell types and are subject to feedback loops. Our biosensors have provided fundamental insights into cellular signalling and can now be used to screen for novel drug-like molecules. This is only possible since we have access to new assays for protein activity.

Our lab has also developed small molecule chemical probes for visualising changes in biological membranes and cellular organelles. Chemistry offers incredible strength and diversity for probing complex microenvironments in a comprehensive manner. Our group has reported new multifunctional probes for measuring changes in chemical environment and local ordering of biological membranes. We have also developed novel probes for measuring dynamics and function of a key cellular organelle, the mitochondria. Mitochondria is a power house of the cell and is critical player in multiple diseases classes. We have developed fluorescent reporters and tracking molecules that are highly photostable and can be used in sensitive cells and tissues including primary cells and human stem cells. This has been applied in tracking and measuring mitochondrial dynamics in stem cell based cellular models of human diseases like Alzheimer's disease.

We take inspiration from nature for developing novel sensors and switches. We have made significant discoveries in natural light-sensing that have shaped the current

understanding of how light is sensed and processed in nature. This work has led us to develop new assays for functional eye-brain regeneration that we are deploying in a collaborative manner. For instance, with Dasaradhi Palakodeti and Jochen Rink (Gottingen), we are using these assays to uncover new regulators of eye and neural patterning and function. Apart from this, we have also discovered novel light sensing modules that enable organisms to sense light independent of their eyes. Our work and the new discoveries made at inStem expand the repertoire of biological sensors available and lays the platform for new technologies where principle of natural light sensors can be harnessed.



Schematic showing the programme initiated by the laboratory. There are two main programmes initiated; 1. Understand the mechanism that drive natural light sensing in regenerative models, 2. Design biosensors to study protein chemistry and organelle dynamics.

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Awards

Praveen Vemula:

Gandhian Young Technological Innovation Award – 2019 By BIRAC-SRISTI (Biotechnology Industry Research Assistance - Society for Research and Initiatives for Sustainable Technologies and Institutions).

Research Talks

Ramaswamy Subramanium:

When curiosity killed the cockroach - Poorna Prajna Research Institute. Plenary lecture. Bangalore, India. July 2018.

Adaptation in Biology - SERB school on Chemical Biology, IISc, Bangalore, India, July 2018.

The EM structure of a bifunctional enzyme - Electron Microscopy Society of India annual meeting, Bhubaneshwar, India, July 2018.

Cockroaches and fish – phenomenology and science in Biology - MACFAST, Thiruvalla, India, September 2018.

Resolution revolution in structural biology - Oxford College, Bangalore *(a meeting of the Indian science Academy)*, October 2018.

Many structures and no drugs - Hong Kong University, Hong Kong, December 2018.

Three talks on **Structural Biology and EM** at Biophysics and Structrural Biology at Synchrotrons, University of Cape Town, Cape Town, South Africa, July 2019.

Structure assisted Drug Discovery - EMBO symposium on Structure assisted Drug discovery, New Delhi, India, February 2019.

Dasaradhi Palakodeti:

Translation regulators in stem cell function and regeneration - Seminar at MBU, IISc, Bangalore, India, April 2019.

Translation regulators in stem cell function and regeneration - DBS seminar at IISER Mohali, India, April 2019.

Dynamic change in tsRNAs regulate cell state transitions - Genome architecture and cell fate regulation conference, Hyderabad, India, December 2018.

Dynamic change in tsRNAs regulate cell state transitions - UK-India Cancer informatics workshop held at Mumbai, India, October 2018.

Praveen Vemula:

Science for solving societal needs: a biomedical research approach - Nirmala College, Muvattupuzha, Kerala, India, June 2019.

Therapeutic and prophylactic nanomaterials for biomedical applications - Dayanand Sagar University, Bangalore, India, April 2019.

Designing therapeutic and prophylactic materials for biomedical applications - Indian Institute of Technology, Kanpur, India, March 2019.

Disease-responsive biomaterials: a novel concept for the treatment of autoimmune and inflammatory diseases - NWNTD-2019, Indian Institute of Technology, Guwahati, India, February 2019.

Prophylactic biomaterials to protect farmers from pesticide-induced neuronal damage - The SPIRITS International Conference, iCeMS, Kyoto, Japan, January 2019.

Disease-responsive biomaterials: a novel concept for the treatment of autoimmune and inflammatory diseases - The SPIRITS International Conference, iCeMS, Kyoto, Japan, January 2019.

Designing therapeutic and prophylactic materials for biomedical for biomedical applications - CSIR-Centre for Drug Research Institute, Lucknow, January 2019.

Prophylactic biomaterials to protect farmers from pesticide-induced toxicity and mortality - St. Xavier's College, Mumbai, January 2019.

Disease-responsive biomaterials: a novel concept for the treatment of autoimmune and inflammatory diseases - International Conference on Advances in Materials Science & Applied Biology (AMSAB 2019), NMIMS University, Sunandan Divatia School of Science, Mumbai, January 2019.

Translating technologies in India, a scientist's perspective - INSA Annual General Meeting & Symposia, December 2018.

Prophylactic technologies to prevent pesticide-induced toxicity and mortality - Bangalore INDIA NANO, Bangalore, December 2018.

Key aspects of science-entrepreneurship in materials research - Higher Education Academy, Karnataka University Campus, Dharwad, November 2018.

An active topical gel to prevent pesticide-induced neuronal dysfunction - NCBS-Sangat Meet, NCBS, Bangalore, October 2018.

Disease-responsive biomaterials for the treatment of autoimmune and inflammatory diseases - Nanobioteck 2018, AIIMS-IIT-Delhi, Delhi, October 2018.

Nanomedicine: from bench to bedside - Nanobioteck 2018, AIIMS-IIT-Delhi, Delhi, October 2018.

Disease-responsive biomaterials: an emerging concept for the treatment of autoimmune and inflammatory diseases - Trinational Conference, MRSTC, CeNS, Bangalore, October 2018.

Translational science: a tale of three (ad)ventures in science-entrepreneurship - Siddaganga Institute of Technology, Tumkur, October 2018.

Disease-responsive drug delivery: an emerging concept for the treatment of autoimmune and inflammatory diseases - 4th International Conference on Translational Research, Goa, October 2018.

Disease-responsive drug delivery: an emerging concept for the treatment of autoimmune and inflammatory diseases - Molecular Biophysics Unit, IISc, Bangalore, September 2018.

Prophylactic technologies to prevent pesticide-induced toxicity and mortality in agriculture farmers - Indo-Brazil Agrotech Workshop, CCAMP, Bangalore, September 2018.

Principles of alternative drug design: Innovative delivery approaches to enhance the efficacy of drugs - Science Academies Lecture Workshop in 'New Frontiers in Chemistry', Indian Academy Degree College, Bangalore, August 2018.

Translational science: a tale of three (ad)ventures in science-entrepreneurship - JSS College of Pharmacy, Mysore, August 2018.

Disease-responsive drug delivery: A novel concept for the treatment of autoimmune and inflammatory diseases - ICAR-IVRI, Bangalore, July 2018.

Akash Gulyani:

Biosensors and probes for cellular dynamics - Regional Centre for Biotechnology, RCB, Delhi-NCR, Invited research lecture at RCB Bio-Imaging School, March 2019.

Visualising cellular dynamics and natural light sensing - Invited talk, Indo-Japan collaboration and discussion meeting NCBS 2019, March 2019.

Sensing across scales: biosensors, fluorescent probes and natural light sensing - Invited talk at Tata Institute for Fundamental Research (TIFR), Bombay, Tata Institute for Fundamental Research-Weizmann Institute joint research meeting (TWIM 2019), January 2019.

Sensing across scales: fluorescent biosensors and probes for cellular dynamics and protein activities - Invited research lecture at the "National Workshop on Fluorescence and Raman Spectroscopy (FCS-2018)", New Delhi, a meeting of the fluorescence society of India, November 2018.

Natural light-sensing and fluorescent biosensors - Invited talk at Department of Physics, Duke University, Durham, USA, October 2018.

Sensing across scales: visualising cellular dynamics and discoveries in natural light sensing - Advances in Science, Engineering and Technology, ASET colloquium TIFR, Tata Institute of Fundamental Research (TIFR) Bombay, August 2018.

Multilayered and dynamically interacting light sensing networks in a fully regenerating flatworm - Invited talk in the plenary session, Gordon Research Conference (GRC) on Photosensory receptors and signal transduction, Italy, March 2018.

Outreach

Ramaswamy Subramanium:

Radio interview on science and society – MacFast, Thiruvalla, 90.4 FM, September 27, 2019.

Attitude, inspiratin and perspiration – National post-doc symposium, CCMB, Hyderabad, October 2018.

Global technology summit – conversations on genome engineering and public policy, December, 2018.

Nobel symposium on the prize in chemistry – directed evolution, Maharanis college, Bangalore, December, 2018.

On why engineers should learn biology – Atria College of Engineering, Bangalore, February 2019.

Member of research impact fund committee – RGC/UGC, HongKong (2018-2020).

Dasaradhi Palakodeti:

Conducted workshop at IISER Mohali on **planarian regeneration for undergraduates** organised by Dr. Sudip Mandal, April 4th -5th, 2019.

Praveen Vemula:

Science for Society: technologies for biomedical applications - Venue : University of Agricultural Sciences, GKVK-Campus, May 2019.

Science for society - TEDx at KIIT University, Bhubaneshwar, March 2019.

Anti-pesticide technology, a boon for farmers - TEDx at Siddaganga Institute of Technology, Tumkur, January 2019.

Akash Gulyani:

Discoveries in natural-light sensing and cellular dynamics - Invited talk at National Science Day 2019, MACFAST (Mar Athanasios College for Advanced Studies Tiruvalla), Kerala, February 2019.

Natural light-sensing and regeneration - InSearch 2019 Jawaharlal Nehru Centre for Advanced Scientific Research. Research talk and outreach session with undergraduate and school students, February 2019.

10

TIGS-CI

Tata Institute for Genetics and Society - Centre at inStem

The Tata Institute for Genetics and Society (TIGS) team seeks to utilise the latest revolutionary advances in genetics to address the pertinent questions in biology that are relevant to India. As a relatively young centre, our current efforts focus on human health and agriculture, and conservation biology is an area we are exploring.

Our largest programme is on mosquitoes as vectors of diseases, such as malaria, dengue and chikungunya. We investigate mosquito biology and population ecology, and explore genetic strategies to either control their populations or their ability to transmit disease. In two collaborative efforts with Amrita University and JNCASR, we explore strategies to combat multi-drug resistant bacterial infections and develop haematopoietic stem cell therapies for monogenic diseases, such as sickle cell anaemia. In agriculture, we work with breeders in agricultural universities in India to enhance specific qualities of rice varieties that have been developed previously through traditional breeding methods.

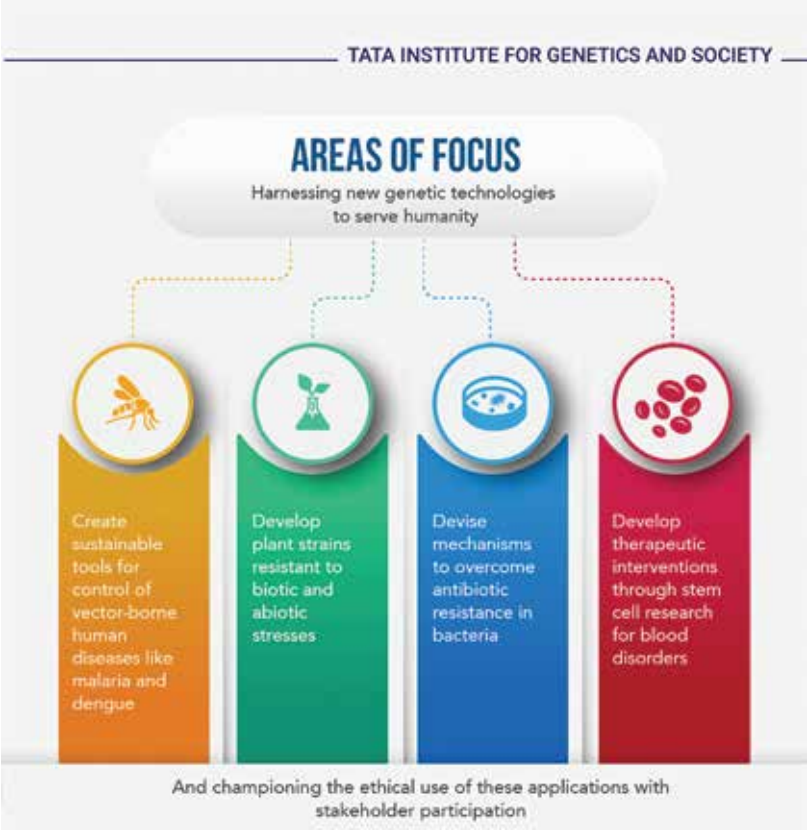
Theme Coordinator:

Suresh Subramani

Global Director, TIGS

New Genetic Solutions to Improve Human Health and Agriculture

TIGS-Centre at inStem (TIGS-CI) was launched in the summer of 2018 and is one of two such centres funded by the Tata Trusts. The other, TIGS-UCSD, is based out of University of California, San Diego. Both centres seek to harness emerging new developments in genetics to serve society and collaborate closely to implement the mission of TIGS. We report here the progress on the current programmes of TIGS-CI, which are also summarised in the figure below.



Active Genetics is a new area of genetic engineering technology that can be used to make targeted changes in the genetic content of an organism, propagate the changes throughout a population in a very fast and efficient way and ensure that almost all the progeny of the population carry the targeted change by bypassing classical Mendelian inheritance mechanisms.

Control of vector-borne human diseases

Mosquitoes are vectors for a long list of viral and parasitic diseases. They are acquiring resistance to traditional pest control methods, such as pesticides and insecticide-treated nets, which are also expensive to deploy in large or remote geographical areas. New genetic strategies to combat malaria include population replacement, which immunises mosquitoes and prevents them from transmitting the malaria parasite, and population suppression, which reduces the mosquito population.

Dr. Baskar Bakthavachalu's team at TIGS is devising a strategy to selectively target only pathogen-infected insect populations. The group aims to disseminate a genetic cargo containing an inactive zymogen into the vector species. When the vector is infected by the infectious agent, the zymogen will be switched 'on', resulting in the death of the infected vector. The team is currently testing the proof-of-concept in Drosophila, with the aim of extending the method to control Flavivirus infection by Aedes aegypti. His team also collaborates with Dr. Vinothkumar Raghunath at NCBS to determine the structure of ribosomes using cryo-electron microscopy.

Dr. Anirudha Lakshminarasimhan's team at TIGS is working towards identifying effector molecules that can impart immunity to mosquitoes when they are exposed to the malaria parasite. The effector gene products would target Plasmodium, or in some cases Anopheles, proteins leading to the blockade of pathogen transmission inside mosquitoes. The selected effector molecules would be expressed using recombinant insect and mammalian cell expression systems, purified and characterised. The genes for the effector molecules would then be modified using site-directed mutagenesis for improved stability and/or oligomerisation. Antibodies with greater stability are anticipated to have higher transmission-blocking efficacy when expressed in mosquitoes.

Dr. Sunita Swain leads the TIGS Insectary operations, which began in October 2018. Her team has established mosquito populations from different parts of the country and would be instituting more Anopheles colonies in the coming year. In collaboration with TIGS Visiting Professor Dr. Subha Srinivasan from the Institute for Bioinformatics and Applied Biotechnology (IBAB) and Dr. J.J.Emerson from UC Irvine, the team has generated a reference genome for an Indian An. stephensi strain and will extend this to additional isolates. A robust, user-friendly database of DNA and protein sequences from malaria-transmitting mosquito populations has been created and is expected to be a valuable resource for academic and government agencies in India. The team has studied DNA sequence variation in coding versus non-coding regions, as well as within introns and exons, and has defined invariant loci suitable for genetic modification.

Dr. Shaibal Dasgupta and Dr. Farah Ishtiaq lead field surveillance of mosquitoes, in collaboration with Dr. K. Jayalakshmi at the Central University of Tamilnadu, to facilitate the analysis of sequence variation, population genetics, structure, gene flow and behavior in island and mainland populations. Dr. Uma Ramakrishnan at NCBS is a collaborator in this work.

Development of new plant varieties

Dr. Venkata Sresty Tavva leads the rice research efforts at TIGS and is working on developing gene editing tools in rice in collaboration with scientists at TIGS-UCSD. They aim to design and construct vectors to test gene editing mechanisms in rice and modify target genes through *(site-directed nuclease)* SDN1-mediated genome editing. The team will also target genes to improve agronomic traits of relevance to local breeders and agricultural institutions, with whom the team hopes to interact and collaborate.

Reversal of antibiotic resistance in bacteria

TIGS collaborates with Amrita University, Kerala, to identify genetic strategies to reverse multi-drug resistance (MDR) in bacteria. Dr. Bipin Nair's team primarily focuses on antibiotic resistance in *Pseudomonas aeruginosa.* They are working towards selecting lysogenic phages that can invade both wild type and clinical regional strains of P.

aeruginosa and deliver the CRISPR-Cas9 genome editing machinery against antibiotic resistance genes. The antibiotic-resistance genes have been identified through gene expression analysis of drug-resistant, clinical regional strains of P. aeruginosa.

Stem cells as models for human disease

TIGS is working towards creating cell culture models and cures for human blood disorders using cultured stem cells of the hematopoietic system. This research is in collaboration with Dr. Maneesha Inamdar, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR).

Dr. Sonia Sen's team at TIGS studies how neural stem cells (NSCs) use spatial and temporal cues to generate neural diversity. NSCs experience unique spatial cues, depending on their position along the anterior-posterior or dorsoventral axis during development. This imparts unique molecular identities to them, and therefore their ability to generate different neuron types. Then temporal cues allow NSCs to generate different neurons over time. The combination of spatial and temporal cues generates all of the diversity of the nervous system. Dr. Sen's group focuses on how spatial identities are established and maintained in NSCs, and how this is integrated with the temporal axis to generate diverse neuronal types. Understanding this will allow their group to reprogram NSCs to reverse engineer specific neuron types. They exploit the genetic versatility of *Drosophila* to understand this process and will explore translation in human brain organoid systems.

Publications

Efficient allelic-drive in Drosophila. Nat Commun. 2019 Apr 9;10(1):1640. doi: 10.1038/s41467-019-09694-w. PMID:30967548
Guichard A, Haque T, Bobik M, Xu XS, Klanseck C, Kushwah RBS, Berni M, Kaduskar B, Gantz VM, Bier E.

A question of lineage. eLife. 2019 May 7;8. pii: e47162. doi: 10.7554/eLife.47162. PMID: 31063134
Sen S.

Awards / Recognition

Baskar Bhaktavachalu – Grant Challenges Explorations (GCE) Award for "Inducible gene drive based approach to control infectious insect vectors" – funded by Sentinels Review Panel (SPR), Program Management Unit, and jointly supported by DBT-BMGF-BIRAC-Wellcome Trust.

Sonia Sen – Ramalingaswami Re-entry fellowship of the Department of Biotechnology for 2018-2019.

Research Talks

Harnessing science to serve humanity – Vison of Tata Institute for Genetics and Society, Lakshmi Mittal Foundation, Delhi, March, 2019.
S Subramani.

Panel discussion on **technological advancements in Agriculture,** Science and Society symposium, organised by NITI Aayog, Office of The Principal Science Advisor to PM, the Lakshmi Mittal and Family South Asia Institute, Harvard University, Vigyan Bhavan, New Delhi, April, 2019.
S Subramani.

11

CSCR

Centre for Stem Cell Research

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

Theme Coordinator:

Alok Srivastava – Head, CSCR

RV Shaji

Saravanabhavan T

Mohankumar M

Srujan Marepally

Asha M Abraham

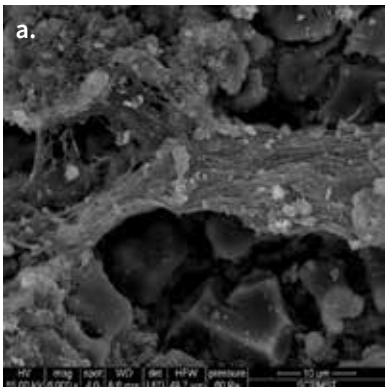
Dolly Daniel

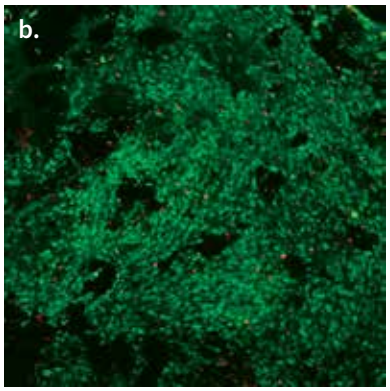
Vrisha Madhuri

Brief outlines of the major thrust thematic areas of research at CSCR:

Musculoskeletal regeneration

This programme is coordinated by Vrisha Madhuri with a large team of clinical and basic scientists including several external collaborators. This group aims to develop novel therapies to address unmet needs of patients with bone, cartilage and muscle disorders. The major focus is on clinical translation related to physis, articular cartilage and bone regeneration. For articular cartilage regeneration small and large animal studies have been completed with differentiated MSCs with either growth factors or miRNA on indigenous scaffolds with successful outcome (DST). There is a new focus on using biomolecules on scaffold for regeneration with in vitro studies completed and ongoing large animal studies for segmental bone defect and osteochondral defects (Indo-Danish, DBT). The continued follow up for pilot human physeal regeneration with culture expanded autologous chondrocytes has shown success at six years and patient recruitment for phase 1 clinical trial is under progress (DHR). This group has also achieved success in physeal regeneration using hydrogel scaffolds in large animal model. A first-of- its-kind pilot study on human bone defect regeneration study with tissue-engineered bone has been completed with a follow up of 2.5 to 4.5 years and further preclinical work is ongoing in the area of bone regeneration using biomolecules. A phase I/II clinical trial, Boost to Brittle Bones, has been initiated in collaboration with Karolinska Institutet, Sweden for the treatment of osteogenesis imperfecta (OI) using foetal liver mesenchymal stem cells (Indo-Swedish, DBT); a parallel study is being conducted for screening genetic heterogeneity in children with OI (ICMR). A new pilot study for the treatment of urinary incontinence using autologous muscle derived cells has been approved (ICMR). Under international collaboration the work on non-invasive manipulation of physeal cartilage and muscle derived stem cell for anal sphincter repair continues.





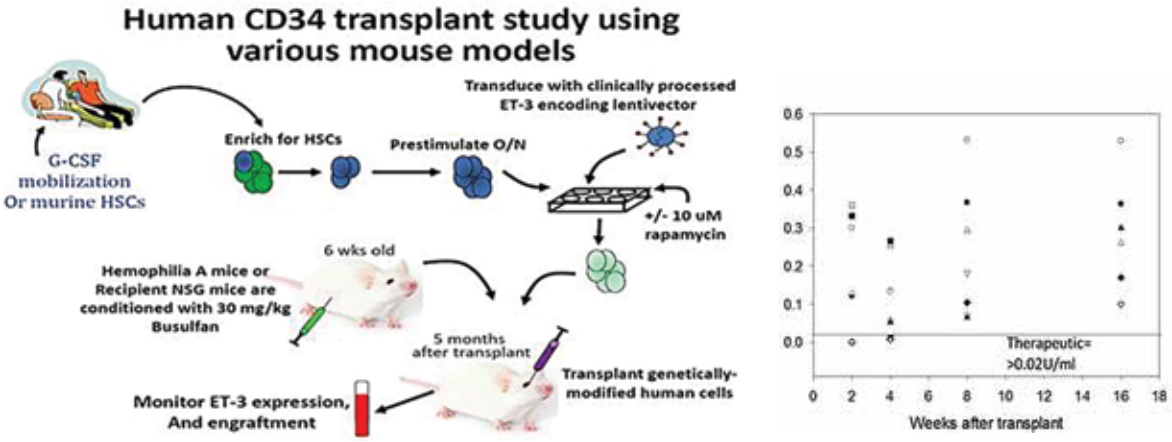
*Tissue engineered bone at day 8 of culture: **a.** SEM image showing a band of clumped mesenchymal stem cells seeded on hydroxyapatite scaffold with good to excellent scaffold and cell to cell interaction; **b.** live dead assay showing more than 90% cell viability (green) on cell-seeded construct with few dead cells shown in red.*

Gene therapy

A major focus of research at CSCR is on gene therapy. Our goal is to capitalise on the recent advances in the world towards gene therapy of monogenic haematological disorders. and make them possible for patients in India. Several scientists and physicians are involved with this work, which is coordinated by Alok Srivastava (AS) and includes R V Shaji (RVS), Saravanabhavan Thangavel (ST), Mohankumar Murugesan (MM) and Srujan Marepally (SrM) at CSCR and several other faculty from CMC, Vellore as well as many external collaborators.

Haemophilia: The development of AAV vector-based gene therapy clinical trial for haemophilia B is the major initial thrust of this programme led by AS. A clinical trial for gene therapy of haemophilia B is being planned. A unique vector (AAV3) and FIX transgene have been designed for this clinical trial, and its functionality has been confirmed in vitro and in vivo. The results are very encouraging in both of these models which sets the stage for clinical studies. However, there have been unanticipated challenges in the plans for GMP production of AAV3. Since then we have explored the option of several other sites *(academic and contract manufacturing organisations)* for getting this AAV production done. We have an in-principle agreement from the GMP facility at the Cincinnati Medical Centre, USA which also has considerable experience in producing vectors for gene therapy trials, of making this vector for us by the end of 2019. If that happens, we will be able to proceed with the clinical trial plans after testing it in the NHP models at the Emory University. Other options are also being explored to see whether this can be achieved sooner.

Apart from this, a novel lentiviral vector-based gene therapy for haemophilia A has also been developed over the last 2 years in collaboration with the scientists at the Emory University, Atlanta, USA (AS) *(Hum Gene Ther. 2018, 29:1183-1201 - see figure below).* A proposal for a phase 1 clinical trial has been reviewed by the CDSCO and is in final stages of review. An agreement has been reached for full freedom to operate in India with this product, if found successful in this trial. In the meantime, the same product was submitted about 6 weeks ago for a phase 1 clinical trial in USA but with a different clinical protocol. This trial has received US FDA approval about 2 weeks ago. This approach is also important to explore for haemophilia as >50% of the patients may be ineligible for current AAV-based gene therapy due to pre-existing anti-AAV antibodies. The concept here is to transplant autologous haematopoietic stem cells (HSCs) ex-vivo transduced with a lentiviral vector carrying the FVIII transgene. Though this approach is well established for several diseases including the major haemoglobin disorders, this is the first such proposal for haemophilia in the world. It is also the first proposal for a clinical trial of gene therapy in India.



To explore other options for gene transfer technologies for haemophilia, work is ongoing for developing a novel ex-vivo gene therapy by targeted integration of FVIII transgene in hematopoietic stem cells through CRISPR-Cas9 technology (MM). Given the expertise for lipid-based gene transfer at CSCR, work is also ongoing on applying this approach to haemophilia through liver-targeted liposomal formulations (SrM). Both cellular and transgenic haemophilia animal models will be used to test these approaches.

An industry collaboration has been established with Intas Pharmaceuticals for the development of rAAV8-hFIX-Padua based gene therapy for Haemophilia B. This work is coordinated at CSCR by Sanjay Kumar. In-vivo efficiency of expression in being evaluated in the transgenic haemophilia mouse models at CSCR.

To improve the current approaches of gene therapy for Haemophilia A, MM is working on a novel ex-vivo gene therapy approach for targeted integration of FVIII in hematopoietic stem cells for the treatment Haemophilia A. A protocol has been developed for effective transfection of Cas9-RNP complex for the targeted integration of transgene in to lineage specific promoter.

Towards developing a novel lipid mediated gene therapy strategy for haemophilia, galactosylated lipid nanocarriers have been developed that can specifically deliver nucleic acids including pDNA, siRNA, mRNA effectively into the liver. Further, safety profiles and therapeutic efficacy are being assessed in Haemophilia B mouse model.

Haemoglobin disorders: Another major thrust of the gene therapy is on the major haemoglobin disorders such as thalassemia and sickle cell disease which are major public health problems in India. Two approaches are currently under development – lentiviral vector-based gene transfer approach which is already being evaluated in animal models *(RVS/AS - details under the NAHD section)* and a novel gene-editing approach using the CRISPR-Cas9 technology for correction of the phenotype of β-thalassemia major and sickle cell disease by altering the expression γ-globin chains through transcriptional modifications (ST/MM) in collaboration with two groups at the University of California, USA through a INDO-US exchange programme.

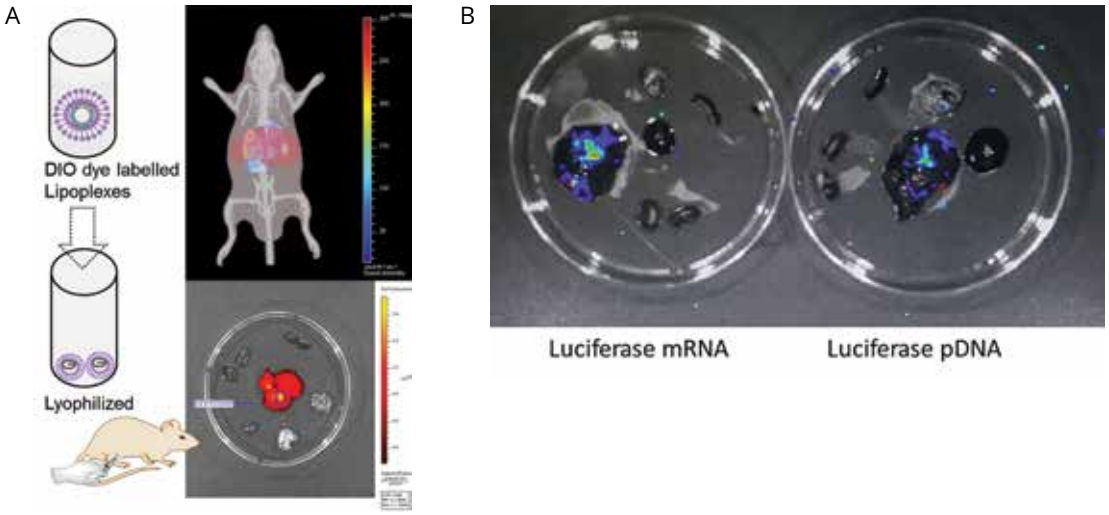


Figure: Biodistribution of GALINAs. Dil labelled GALINAs accumulated specifically in liver (A) and liver specific mRNA and pDNA expression was found (B).

Other diseases: Using CRISPR/Cas9 gene editing tools preclinical studies are also ongoing to develop gene correction in Wiskott-Aldrich syndrome (WAS). Gene editing tools and strategies are being tested for the targeted integration of the WAS transgene in the hematopoietic stem cells. Newer areas of research are being established to assess anti-tumor functions of NK cells, γδ T cells and αβ T cells with the specificity and robustness of Chimeric Antigen Receptors (CARs). The area of immune cell therapy is being coordinated by Sunil Martin.

Cellular reprogramming and its applications - disease modelling and haplobanking

The area of cellular reprogramming technology is coordinated by R. V. Shaji at CSCR. This technology is now being applied to two areas of translational research, disease modelling, and haplobanking.

Generation of iPSCs for disease modelling of haematological diseases: RVS lab has been working on establishing iPSC-based disease models for haematological diseases. His group successfully generated iPSCs from patients with Fanconi anaemia and haematopoietic cells generated from these iPSCs mimic Fanconi anaemia disease in culture. Currently, this cellular system is being used for understanding the molecular basis of Fanconi anaemia. Recently, this group generated iPSCs from a patient with Diamond Blackfan anaemia, a genetic disease that cause ineffective erythropoiesis.

A major translational effort has also been initiated towards establishing a "haplobank" for generating iPSCs from individuals homozygous for HLA haplotypes. This area is coordinated by Dolly Daniel from Department of Transfusion Medicine and Immunohematology, CMC, Vellore and R. V. Shaji from CSCR. Liaising with the stem cell registry DATRI, and also including patients/donors HLA typed in CMC, to date 237 donors representing the top 20 haplotypes have consented and samples were drawn for iPSC production. Processes are in place for collection of donor samples and protocols for the derivation of iPSCs in a GMP facility have been standardised. HIPAA and HITEC compliant biobanking system has been installed and is in use. So far iPSC lines from 10 donors have been generated. Out of these, the iPSC lines from 5 donors were generated in the GMP facility. Clones with high genome stability have been generated and have been fully characterised.

Novel approaches to haematological diseases (NAHD) programme

A major enhancement of some of the existing efforts at CSCR have come through the programme on Novel Applications in Haematological Diseases (NAHD) under the multi-institutional Indo-Japan programme on Accelerating Applications of Stem Cells to Human Diseases (ASHD). The NAHD segment of this programme is carried out at CSCR / CMC and has the following components:

I. Clinical trial for gene therapy of Hemophilia B: Details of this project are provided under the "Gene Therapy" section of this report.

II. Standardisation of anti-AAV antibody assays: The goal is to standardise assessment of anti-AAV antibody through different assays to allow appropriate selection of patients for gene therapy. This work is coordinated by Asha M Abraham along with Hubert Daniel, and Rajesh Kannangai from Department of Clinical Virology, CMC, Vellore and Sanjay Kumar and Alok Srivastava from CSCR. It is being done in collaboration with the University of Florida, USA. Both binding and neutralising antibodies are being assessed through the whole capsid and serotype-specific peptide ELISAs and transduction inhibitions

assays (TIA), respectively. The whole capsid and peptide ELISAs have been standardised for AAV 3, 5 and 8. TIA by mCherry based flow-cytometry had been standardised for AAV 3 and 5. 50 hemophilia A or B and 50 healthy individual sera were tested for whole capsid and specific peptide antibodies. While about 50% of patient samples tested so far are positive for AAV3 antibodies, 18% of individuals are negative for antibodies to AAV 3, 5, and 8.

III. Late pre-clinical research - lentiviral & genome editing approach for thalassemia and sickle cell disease: This project aims to evaluate lentiviral vectors for developing gene therapy for the major haemoglobin disorders. This is coordinated by R V Shaji and Alok Srivastava. In collaboration with Emory University, lentiviral vectors have been generated for gene therapy of haemoglobinopathies. These vectors have been evaluated and those which high efficiency for the expression of beta globin gene have been identified for testing in mouse models. Recently, a lentiviral vector has been developed that can downregulate the expression of BCL11A only in erythroid cells, and this vector is found to increase the expression of gamma globin gene significantly. Further experiments are being carried out in mouse models and in the cultured erythroid cells from patients with haemoglobinopathies.

Another important component of this programme is the gene editing approach to reactivation of fetal haemoglobin production. This work is being carried out by Saravanabhavan Thangavel and Mohankumar Murugesan using the CRISPR-Cas9 technology in collaboration with the University of California. For beta hemoglobinopathies, two different strategies of disease reversal are being developed. One strategy involves the correction of disease-causing mutation, the second strategy is to mimic the naturally existing beneficial mutations for reactivation of fetal gamma-globin activation. Towards these, reagents have been developed and conditions have been optimised for genetic manipulation in primary hematopoietic stem cells. Currently ST & MM labs are testing hemoglobin expression pattern in the erythroid cells differentiated from manipulated HSPCs.

To compensate the deficient expression of Wiskott-Aldrich Syndrome protein, transgene constructs have been developed for targeted integration in HSPCs. Currently work is ongoing on the ways to overcome the cell toxicity related to the process. There are naturally existing beneficial mutations that provide resistance against HIV infection. These beneficial mutations have been introduced in the HSPCs and consequence of editing this locus on the HSPCs is being tested. A platform is also being developed for maintaining the stemness of HSPCs during ex vivo culture and currently testing the ex vivo expanded cells for the engraftment and differentiation in NSG mouse.

IV. Early pre-clinical research for hemoglobin / erythroid disorders: The aim is to create disease models for two monogenic erythroid disorders, Diamond Blackfan Anemia (DBA) and Congenital Dyserythropoietic Anemia (CDA), by creating mutations in the associated genes by CRISPR/Cas9. The target genes have been successfully disrupted by CRISPR/Cas9. Currently, a novel approach to introduce biallelic mutations along with selection markers to screen colonies for target mutations is being established. Methods to differentiate iPSCs to haematopoietic progenitor to erythroid cells have also been developed. An iPSC line that expresses Cas 9 from AAVS1 site in doxycycline individual manner has been developed.

V. HAPLOBANKING - Bank of iPS cells from individuals with homozygous HLA haplotypes: This project is aimed at creating a bank of iPSCs derived from individuals homozygous for the most common HLA haplotypes in the Indian population. Details of this project are provided under the *"Cellular Reprogramming and Its Applications"* section of this report.

VI. Control of thalassemia and sickle cell disease – Creating a model for India: This programme is led by Kuryan George and Shantidani Minz along with several other colleagues from the departments of Community Health, Haematology and Transfusion Medicine and Immunohaematology at CMC, Vellore. A collaboration was established with the Department of Health and Family Welfare and the National Health Mission of the Govt. of Odisha. This is a unique programme in terms of scale and complexity in this field in the world. Six districts have been identified to implement the first phase of this programme. Towards increasing capacity and capability for treatment of major haemoglobin disorders in Odisha, workshops are being arranged at different levels (State / Regional / District levels) for doctors / other healthcare workers of Odisha.

Publications

First report of a tissue-engineered graft for proximal humerus gap non-union following chronic pyogenic osteomyelitis in a child. Journal of Bone and Joint Surgery Case Connector – Accepted for publication, July, 2019.
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Patents

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Research Talks

Vrisha Madhuri:

Genetic heterogeneity in osteogenesis imperfecta in Indian children - Asia Pacific Paediatric Orthopaedic Society 2019, Incheon, South Korea, April 2019.

Research in orthopaedics - Paediatric Orthopaedic Society of India 2019, Mumbai, India, January 2019.

Alok Srivastava:

Gene therapy for haemoglobin disorders – Where are we?– 11th National Haematology Update, All India Institute of Medical Sciences, New Delhi, India, April 2018.

Gene therapy for haemoglobin disorders – Symposium on Hemoglobinopathies, CSIR-Institute of Genomics & Integrative Biology, New Delhi, India, March 2018.

Managing haemophilia – moving from protein to gene replacement – Symposium on Gene Therapy in Modern Medicine, Loma Linda University, California, USA, November 2018.

Replacement therapy for haemophilia – from protein to gene – International Academy for Clinical Haematology (IACH) – 1st Annual Meeting, Paris, France, September 2018.

RV Shaji:

Molecular basis of Fanconi anaemia - PGIMER, Chandigarh, India, January 2019.

Applications of pluripotent stem cells - 3rd annual cell and gene therapy symposium, Centre for Stem Cell Research, Vellore, India, September 2018.

Application of NGS in inherited haematological diseases - 7th Annual MPAI Conference, ACTREC, Mumbai, India, January 2019.

iPSCs for disease modelling - VIT, Vellore, India, October 2018.

Molecular basis of Fanconi anaemia - Haematocon 2018, Kochi, India, October 2018.

Srujan Marepally:

Novel strategies for cationic lipid enabled CRISPR/Cas9 based genome editing in hematopoietic cells - 3rd Annual Cell and Gene Therapy Symposium, Centre for Stem Cell Research, Vellore, India, September 2018.

Saravanabhavan Thangavel:

Gene editing approaches for the treatment of Beta hemoglobinopathies - 3rd Annual Cell and Gene Therapy Symposium, Vellore, India, September 2018.

Mohankumar Murugesan:

Therapeutic genome editing for beta hemoglobinopathies - BioMET 2018, Vellore Institute of Technology, Vellore, India, July 2018.

Therapeutic genome editing for beta hemoglobinopathies - 3rd Annual Cell and Gene Therapy Symposium, Vellore, India, September 2018.

Outreach

Human induced Pluripotent Stem Cells (iPSC) workshop: Centre for Stem Cell Research (CSCR) conducted the second human induced pluripotent stem cells (iPSC) workshop from 18th to 23rd February, 2019. The workshop was designed to provide better iPSC culture practice and train the researchers to translate the techniques in their own laboratories. The workshop involved hands-on-training and guest lectures by invited experts in the field.

Annual cell and gene therapy symposium: CSCR has been organising an annual symposium on Cell and Gene Therapy for the last 3 years. The aim of this meeting is to provide a platform for scientists and physicians working in this field of research to come together and discuss the advances in the field. The 3rd Annual Cell and Gene Therapy Symposium was held on 6th and 7th of September, 2018. Over 120 scientists/physicians from 46 institutions attended this meeting including those from 3 industry groups and which had 9 international speakers. The next annual symposium is scheduled on 5th and 6th of September, 2019.

Awards

Vrisha Madhuri: Elected National Delegate for APPOS.

R V Shaji: Wellcome-DBT India Alliance Senior Research Fellowship, 2019.

Saravanabhavan Thangavel and Mohankumar Murugesan: Awarded Indo-U.S. Genome Engineering / Editing Technology Initiative Fellowship.

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Research Development Office (RDO)

Research at the Bangalore Life Science Cluster which includes NCBS, inStem and C-CAMP, 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.

Over the course of the last nine years, the RDO has supported the diverse needs of the campus in fundraising, grants management and contract negotiation for research funding from funding agencies, corporate sources and charitable organisations.

Generous funding from the Government has been invaluable in establishing large institutional programmes on campus such as the Centre for Chemical Biology and Therapeutics (CCBT), the Centre for Neurodevelopmental Synaptopathies (CNS), the Accelerator Programme for Discovery in Brain Disorders Using Stem Cells (ADBS), the Bangalore Life Science Cluster for Multiscale Basic and Applied Research in the Biological Sciences (B-LIFE), the Programme on Chemical Ecology, the National Mouse Research Resource (NaMoR), and the Macromolecular Crystallography and Scattering Facility. The RDO manages all these large programmes.

The campus has also invested considerable effort into developing a mixed funding portfolio including charitable funding to complement funding from Government and international grants on campus. A recent successful example of a multi-institutional programme benefiting from such mixed funding is the "Accelerator Programme for Discovery in Brain Disorders using Stem Cells (ADBS)" at NCBS and inStem, with institutional collaborations with NIMHANS. This programme is jointly supported by the Pratiksha Trust and DBT. Recently, the Pratiksha Trust has extended its support at NCBS and inStem for a further three years.

Generous funding from the TTK Prestige Group has enabled supporting our vision of "Beyond Boundaries" at NCBS and inStem which in addition to giving significant boost to the Campus Fellows Programme, has enabled us to support International researchers at BLiSC and also institute the "TT Narasimhan travel awards" at NCBS and inStem for supporting students and postdoctoral fellows to attend international conferences and workshops.

Through the generosity of our philanthropic partners the Endowment Fund was initiated in 2016 for research, training, innovation and outreach.

Work at the RDO is made possible by a dynamic and professional team who is committed to offering several key services to the campus at the boundaries of science, management and outreach. We look forward to a rewarding journey further ahead for the RDO, supporting campus research funding and the Endowment Fund.

Vineetha Raghavan

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Communications and Outreach at inStem

As scientists, we recognise that our role extends beyond our labs. inStem organised and participated in various outreach events in an effort to engage with young minds, and to make science more approachable. The philosophy of the institute has been to try and reach the youngest audiences and help spark their curiosity for science.

One rather successful effort has been the exhibition on **"Lab Culture I"** from June to August 2019, which was organised in conjunction with the Museum and Field Stations Facility. Since the start of the exhibition, the campus has hosted over 900 students from 16 schools, including several government schools from in and around Bangalore and 3 colleges. This interactive exhibition worked to explain basic scientific concepts behind the exhibits and the student volunteers communicated with the visiting students in Kannada, Hindi and English. In addition to this, we had two curated events, a discussion on STEM vs STEAM in higher education and a conversation with scientists, where audiences could interact with students and scientists that were involved in the exhibition.

As a follow up, **Lab Culture II** was inaugurated in September 2019 and we hope this will also attract students to learn about and enjoy our activities.

The campus art club organised an, **"art jam"** event in September 2019, the theme of which was, "through the microscope". This event was held at the inStem atrium and attracted over 50 participants, most of them young children. The art that was on display was truly spectacular, particularly from the younger participants.

The **Open Science Day** *(an annual campus-wide event)* was held in November 2018. The campus hosted over a thousand school children from 15 schools and they were treated to lectures and demonstrations by the students and faculty.

The **4th India International Science Festival** was held in Lucknow in October 2018. Several students and faculty from inStem attended this and discussed their work with school children and other members of the public. The interactive demonstration of the regenerative potential of Planaria, where children engaged in simple dissections, was particularly well received.

Another example of a popular campus-wide science outreach program is the **BLiSC Science Café** that brings campus scientists to the public to speak about their work in informal settings, in and around Bangalore and other cities. Several inStem faculty have participated in these events.

All in all, these outreach efforts have resulted in increased interest in our campus, particularly by high school and college students. Over the past year we have hosted more than 500 students from schools, colleges and other institutes across India. More

information about these and other outreach efforts coordinated through the BLiSC Communications Office is provided below.

BLiSC Communications Office

The BLiSC Communications Office supports inStem by providing communications counsel, support, and services to augment the institute's presence through various channels and engagement platforms. An updated, online repository of publications, popular science articles, and news reports of inStem research is maintained. In addition, the Comms. Office has written and designed articles, while nurturing publicity and growing reach via our social media channels and outreach initiatives.

Public & School Engagement: The Cluster's popular sci-outreach programme, the BLiSC Science Café, brings scientists to the public to speak at different social venues in the city.

The **Jigyasa Project** is a BLiSC initiative that seeks to be more audience-inclusive by connecting with non-English speakers. Partnering with the Mandram organisation, we curated a day of science talks in regional languages — namely Tamil, Kannada and Hindi— for our December 2018 edition.

Citizen Matters: *http://bengaluru.citizenmatters.in/scientists-to-talk-in-kannada-and-tamil-on-genetics- at-jigyasa-29878*

Sci-Entertainment: This past year turned our focus onto the area of sci-art and sci-entertainment – looking at new ways to represent science through performances, exhibits, and screenings.

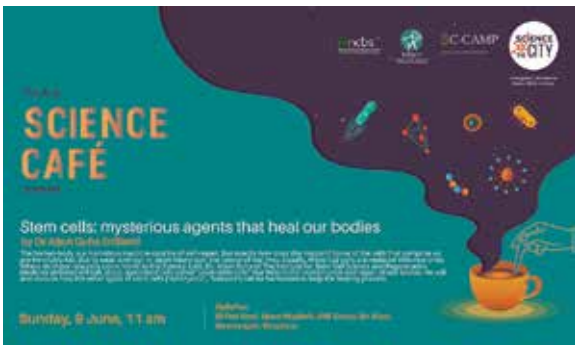
We have screened science-themed films like **"Love & Bananas"** *(elephant rescue in Thailand)*, **"Wild Karnataka"** *(on Karnataka's rich biodiversity),* and **"SuryaGanga"** on water conservation and clean energy. SuryaGanga is being screened in concert with the culmination of the *Swach Bharat initiative* and features a prominent panel which will discuss energy issues against the backdrop of the initiative.

We have seen the popularity of theatre in the city and used it to our advantage seeking out science-theme plays to host on the campus. This creates a bridge of exchange between the campus and the public using the common interest of theatre. This year we have:

- *Songs from Snakes* from the Barefoot Theatre Company, Delhi
- Nilanjan Choudhury's *The Square Root of a Sonnet (The Strange History of Black Holes)*
- *The Vijay Padaki Theatre Festival* by Bangalore Little Theatre
- *Five plays by Stagecraft* - In letter and spirit, and 4 other shorts

Workshops: The Communications Office of the Bangalore Life Sciences Cluster organised a workshop titled **"The Craft of Science Communication"** for its campus members in May this year– the first of its kind designed and executed entirely in-house.

The workshop, comprising five distinct modules spread over five days, aimed to apprise the participants of various aspects of science communication. The first module was an introduction to science communication, followed by sessions on storytelling, written communication, visual communication, and verbal communication. Each module had its own instructor, and the workshop saw a total of 65 registrations, with an average turnout of 20 people per module.



Clockwise from top: (i) Students and Teachers at the Lab Culture Exhibit, May 2019 (ii) The Jigyasa Project (iii) Dr. Dasaradhi Palakodeti with students at the 4th India International Science Festival, Lucknow (iv) Wild Karnataka film screening (v) BLiSC Science Cafe (vi) Open Science Day, November 2018 (vii) The "art jam" event, September 2019

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inStem Investigators

Apurva Sarin, *Senior Professor & Director*
Colin Jamora, *Professor/Investigator*[1]
Dasaradhi Palakodeti, *Associate Investigator*
Srikala Raghavan, *Associate Investigator*
Praveen Kumar Vemula, *Associate Investigator*
Minhaj Sirajuddin, *Assistant Investigator*
Sunil Laxman, *Assistant Investigator*
Tina Mukherjee, *Assistant Investigator*
Dhandapany Perundurai, *Assistant Investigator*
Akash Gulyani, *Assistant Investigator*
Arjun Guha, *Research Investigator*
Ravi S Muddashetty, *Research Investigator*
Shravanti Rampalli, *Research Investigator*

VISITING FACULTY

Mahendra S Rao (NYIRM, New York), *Collaborative Science Chair*
Ashok Venkitaraman (University of Cambridge), *Collaborative Science Chair*
Suresh Subramani (University of California, San Diego), *Collaborative Science Chair*
James Spudich (Stanford University), *Collaborative Science Chair*
Sivaraj Sivaramakrishnan (University of Minnesota), *Visiting Faculty*
Ramaswamy S (Purdue University, USA), *Visiting Professor*
Siddharthan Chandran (University of Edinburgh), *Collaborative Science Chair (until March 2019)*
Peter Kind, (University of Edinburgh), *Collaborative Science Chair (until March 2019)*

1 Joint Appointment with IFOM (Milan, Italy)

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inStem Leadership Committees

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Prof. Apurva Sarin, *Director, inStem, Bengaluru*
Dr. Alka Sharma, *Advisor &Scientist G, DBT, New Delhi*
Mr. B. Anand, *AS & FA, DBT, New Delhi*
Mr. Chandra Prakash Goyal, *Joint Secretary (Admin), DBT, New Delhi*
Prof. K. Vijay Raghavan, *Principal Scientific Adviser to the Govt. (Signatory to MoA)*
Dr. Kiran Mazumdar Shaw, *CMD, Biocon India Ltd., Bengaluru (Signatory to MoA)*
Prof. H. Sharat Chandra, *Hon. Director, Centre for Human Genetics (Signatory to MoA)*
Prof. Goverdhan Mehta, *Former Director, IISc & CSIR Bhatnagar Fellow, Bengaluru (Signatory to MoA)*
Prof. P. Balaram, *Molecular Biophysics Unit, IISc, Bengaluru (Signatory to MoA)*
Prof. Jyotsna Dhawan, *Chief Scientist, CCMB, Hyderabad (Signatory to MoA)*
Prof. Satyajit Mayor, *Centre Director, NCBS, Bengaluru (Signatory to MoA)*
Prof. S. Ramaswamy, *Visiting Professor, inStem, Bengaluru (Signatory to MoA)*
Dr. J. V. Peter, *Director, CMC, Vellore*
Mr. Pawan Kumar Pahwa, *Head-Admin & Finance, inStem, Bengaluru (Non member Secretary)*

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Prof. Alok Srivastava, *Head- CSCR & CMC Vellore*
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Dr. B S Ramakrishna, *Director, SIMS Institute of Gastroenterology, Chennai*

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Mr. Pawan Kumar Pahwa, *Head-Admin & Finance, inStem, Bengaluru – Member Secretary*

NON-ACADEMIC STAFF

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Sreenath B. A., *Admin Officer (Purchase)*
Nagaraja B. S., *Officer on Special Duty*
Shrikant Bhat, *Junior Management Assistant*
Raju B. Verma, *Junior Management Assistant*
Valsala Neyyan, *Administrative Assistant*
Shobha R., *Assistant Administrative Officer*
Sunitha R., *Project Assistant (Admin)*
Shobha B. N., *Project Secretary*
Supriya N., *Project Secretary*

SCIENTIFIC & TECHNICAL STAFF

Sai Sudha, *Scientist D*
Rajesh R., *Engineer D (System Administrator)*
Anand Kumar V., *Engineer D (Electrical)*
Chakrapani, *Junior System Administrator*

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inStem

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