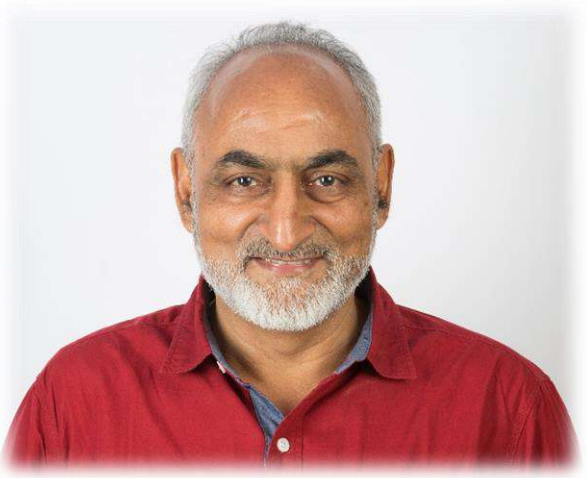
Annual Report

2018-19

CSIR-Centre for Cellular and Molecular Biology
Hyderabad

CONTENTS

प्राक्कथन



अत्यंत हर्ष और गर्व के साथ मैं सीसीएमबी की प्रगति के सभी आयामों को समाहित करते हुए आपके समक्ष वर्ष **2018-19** की वार्षिक रिपोर्ट प्रस्तुत कर रहा हूँ।

सीएआईआर- सीसीएमबी अत्याधुनिक तकनीकों का उपयोग करके आधुनिक जीव विज्ञान के विभिन्न क्षेत्रों में मौलिक अनुसंधान करता है । इन क्षेत्रों में डेवलपमेंटल बायोलॉजी व एजिंग, संरचनात्मक जीवविज्ञान, , जिनोमिक्स व स्वास्थ्य, कोशिका व स्टेम सेल जीवविज्ञान, वनस्पति व सूक्ष्म जीवविज्ञान, वन्यजीव संरक्षण व पारिस्थितिकी शामिल हैं । जीव विज्ञान में आधरभूत अनुसंधान के अतिरिक्त जीवन प्रक्रियाओं की विविधता पर बुनियादी सवालों के उत्तर देने के साथ साथ सीसीएमबी बहुत सक्रिय रूप से कौशल विकास, विभिन्न रूपों में उद्यमिता का समर्थन तथा बैठकों, वैज्ञानिक परामर्श व आउटरीच कार्यक्रमों के माध्यम से समाज में हितधारकों के साथ जुड़ता है । मैं इन सभी पहलुओं पर सक्रिय होने के लिए संतुष्ट हृदय से सीसीएमबी की सराहना करता हूँ ।

आज जिनोमिक्स एक ऐसा उभरता हुआ क्षेत्र है, जो जीवविज्ञान, जैव-प्रौद्योगिकी तथा स्वास्थ्य सेवा के क्षेत्रों की ओर अभिमुख है । सीसीएमबी ने अपनी पूर्व उपलब्धियों को ध्यान में रखते हुए प्रमुख जिनोमिक्स कार्यक्रमों में अपनी प्रमुख भूमिका निभायी है । गत वर्ष, सीसीएमबी ने अपनी मौजूदा जिनोमिक्स सुविधाओं का प्रौद्योगिक उन्नयन, क्षमता निर्माण और विस्तार भी किया है, जिसमें अगली पीढ़ी के अनुक्रमण सीक्वेंसिंग (**NGS**), बिग डाटा मैनेजमेंट और विश्लेषण सुविधा शामिल है । हाल ही में सीएसआईआर प्रयोगशालाएँ - सीसीएमबी और आईजीआईबी ने छह महीने की अवधि में व्यक्तियों के पूरे **1000** जीनोम अनुक्रमण को पूरा करके इस क्षेत्र में अपनी क्षमता और विशेषज्ञता सिद्ध की है । इस क्षेत्र में हमारी क्षमता और संसाधनों को शिखर तक ले जाने और इसका लाभ उठाने के लिए निकट भविष्य में कई बड़े शोध कार्यक्रमों की योजना बनायी जा रही है ।

कोशिका जीवविज्ञान आरंभ से ही सीसीएमबी की आधारशिला रहा है । हमने उसका लाभ उठाकर कोशिका जीव विज्ञान के उभरते क्षेत्रों में नई गतिविधियों की शुरुआत की है । मूल और अनुप्रयुक्त जीवविज्ञान में स्टेम सेल तकनीक अपने विभिन्न अनुप्रयोगों के साथ एक प्रमुख उपकरण है । **CCMB** ने ऑर्गेनॉइड - आधारित बुनियादी और अनुप्रयुक्त अनुसंधान शुरू किया है जो दवा की खोज के कार्यक्रमों को तेज करने में सहायता कर सकता है और अनुसंधान में पशु मॉडल के उपयोग को बहुत हद तक प्रतिस्थापित कर सकता है।

हमने इस वर्ष सीसीएमबी में कॉमन रिसर्च एंड टेक्नोलॉजी डेवलपमेंट हब (**CRTDH**) के पॉच और अटल इन्क्यूबेशन सेंटर (**AIC**) के एक वर्ष पूरे किए । युवा उद्यमियों और स्टार्ट अप्स को प्रोत्साहित करने के हमने अपने इन प्रयासों को पोषित किया है । एआईसी और सीआरटीडीएच दोनों ने ही कई तरीकों से युवा उद्यमियों का सफलतापूर्वक समर्थन किया है । इसमें सीसीएमबी, मार्केटिंग, फाइनेंसिंग, **IP** के साथ संरचनात्मक सुविधाओं तक पहुँच प्रदान करना और विषय विशेषज्ञों और उद्योग प्रमुखों द्वारा परामर्श देना शामिल है ।

हमारी विभिन्न प्रगतियों में से एक, जिसको लेकर हम बहुत उत्साहित हैं कि सीसीएमबी के स्टाफ और हमारे पीएचडी कार्यक्रम के लिए सर्वोत्तम अध्येता के रूप में चुने गए हमारे कुछ छात्रों ने उद्यमशीलता में गहरी रुचि दिखाना शुरू कर दिया है और समाज से जुड़े अनुसंधान समस्याओं का समाधान किया है । समाज से जुड़ी अनुसंधान

समस्याओं का समाधान प्रस्तुत किया है। हमने प्रौद्योगिकियों के सह-विकास के लिए उद्यमियों के साथ सहभागिता भी शुरू की है। इन सहकार्यों में, सीसीएमबी शुरुआत में वैज्ञानिक विशेषज्ञता और सुविधाएँ प्रदान करेगा जबकि उद्यमी बड़े पैमाने पर व्यवसायीकरण को और बढ़ावा देंगे।

ऐसा ही एक अनुप्रयोग–उन्मुख कार्यक्रम क्लीन मीट प्रोग्राम है जिसे हमने नेशनल रिसर्च सेंटर ऑन मीट (NRCM) के साथ शुरू किया है। इस कार्यक्रम में हम अब प्रौद्योगिकी को विकसित करने और इसे व्यावसायिक रूप से आगे बढ़ाने के लिए औद्योगिक भागीदारों को शामिल करेंगे।

सीसीएमबी जनता के लिए किफायती मूल्य पर असामान्य बीमारियों के लिए उच्च स्तर की तक नैदानिक सेवाएँ प्रदान करता है। इस वर्ष, हमने एनजीएस आधारित डायग्नोस्टिक्स को जोड़ा है,जो निदान के क्षेत्र में नवीनतम अत्याधुनिक तकनीक है। हमने सरकारी और निजी अस्पतालों के साथ और मुख्य रूप से सरकारी अनुसंधान संगठनों जैसे सेंटर फॉर डीएनए फ़िंगरप्रिंटिंग एंड डायग्नोस्टिक्स, हैदराबाद के साथ मिलकर इन गतिविधियों को और अधिक प्रभावशाली बनाया है। हमने बायोसिमिलर और हर्बल दवाओं के लिए नियामक प्रक्रिया को सुविधाजनक बनाने के लिए भारतीय फार्माकोपिया आयोग (IPC) के साथ भी हाथ मिलाया है। यह IPC की प्रमुख आवश्यकताओं में से एक है जहाँ सीसीएमबी का उद्देश्य संयुक्त कार्यक्रम के माध्यम से अपना सर्वाधिक योगदान देना है।

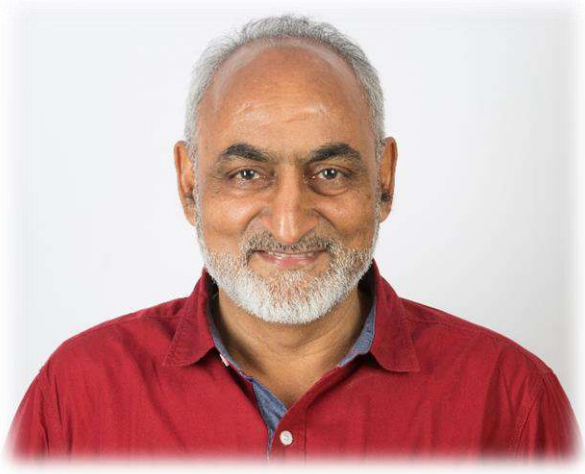
उपर्युक्त कुछ गतिविधियों के साथ, इस वर्ष की रिपोर्ट सीसीएमबी में होने वाले रोचक और रोमांचक विज्ञान की झलक देती है। युवा संकाय सदस्यों में शामिल करते हुए एक नयी पहल के साथ सीसीएमबी, समाज की अपेक्षाओं पर खरा उतरता हुआ और शायद उससे भी आगे.... आधुनिक जीव विज्ञान, जैव प्रौद्योगिकी और स्वास्थ्य सेवा की सीमाओं पर चुनौतियों का समाधान करने के लिए पूरी तरह से आबद्ध है और तैयार है।

राकेश कु मिश्र

राकेश कु मिश्र

निदेशक

FOREWORD



With great pleasure and privilege I present you the Annual Report 2018-19, summarizing our developments at CCMB during this period.

CSIR-CCMB carries out fundamental research in multiple areas of modern biology using cutting edge technologies. These areas include Developmental Biology and Aging, Structural Biology, Genomics and Health, Cell and Stem Cell Biology, Plant and Microbe Biology, Wildlife Conservation and Ecology. In addition to basic research in biology and addressing fundamental questions on variety of life processes, CCMB also very actively carries out skill development, entrepreneur support in various forms, connects with multiple stakeholders in society through the many meetings, scientific interactions and outreach programmes. I can commend CCMB with great satisfaction for being active at all of these frontiers.

Genomics is one of the emerging areas that converges various aspects of biology, biotechnology and healthcare. CCMB has played prominent role in major genomics programmes in the country keeping up with its previous achievements. During the last year, CCMB has also made major capacity building, technology upgradation and expansion of its existing genomics facilities, which includes Next Generation Sequencing (NGS), Big Data Management and analysis facility. More recently CSIR labs – CCMB and IGIB – accomplished 1000 Whole Genome Sequencing of individuals in six months time to demonstrate the capacity and expertise in this area. A number of large research programmes are being planned for near future to capitalize on our capacity and resources in this area.

Cell biology has been CCMB's strength from its inception. We have built upon that advantage, and initiated new activities in emerging areas of cell biology. Stem cell technology is a major tool with multiple applications in basic and applied biology. CCMB has initiated organoid-based basic and applied research that might aid accelerating drug discovery programmes, and substitute the usage of animal models in research to a great deal.

We completed 5 years of Common Research and Technology Development Hub (CRTDH) and 1 year of Atal Incubation Centre (AIC) at CCMB this year. We have nurtured these initiatives to promote young entrepreneurs and start ups. Both AIC and CRTDH have successfully supported young

entrepreneurs in multiple ways; this includes providing access to infrastructural facilities at CCMB, marketing, financing, IP, networking with and mentoring by subject experts and industry leaders.

One of the developments that we are very excited about is that CCMB staff, and in particular students - some of the best brains selected for our PhD programme - have started showing keen interest in entrepreneurship and addressing research problems of societal relevance. We have also initiated collaboration with entrepreneurs to co-develop technologies. In these collaborations, CCMB will provide scientific expertise and facilities in the beginning while the entrepreneurs will scale up and commercialize.

One such application-oriented prorammes is the Clean Meat Programme that we have initiated in collaboration with National Research Centre on Meat (NRCM). With our advances in this programme, we will now involve industry partners for developing the technology further and pursue it commercially.

CCMB provides high end diagnostic services for specific diseases at affordable costs to public. During this year, we have added NGS based diagnostics, latest cutting edge technology in the field of diagnostics among its activities. We have joined hands with government and private hospitals and more importantly government research organizations like Centre for DNA Fingerprinting and Diagnostics, Hyderabad to jointly take these activities to a greater impact. We have also joined hands with Indian Pharmacopeia Commission (IPC) to facilitate regulatory processes for biosimilars and herbal drugs. This is one of the major unmet requirements of IPC where CCMB aims to contribute in a major way through the joint programme.

In addition to the few activities mentioned above, this year's report gives a glimpse of the interesting and exciting science that is happening in CCMB. With newer initiatives, and younger faculty members joining, CCMB is all set to deliver – as per and even beyond – the expectations of the society, and address challenges at the frontiers of modern biology, biotechnology and healthcare.

Rakesh Kumar Mishra
Director

CHARTER

The Centre for Cellular and Molecular Biology (CCMB) is one of the constitutent national laboratories of the Council of Scientific and Industrial Research (CSIR), New Delhi, the premier multi-disciplinary research organization in the country funded by the Government of India. It was set up formally in April, 1977

The objectives of the Centre are:

a) To conduct research in frontier and multi-disciplinary areas of modern biology and to seek potential applications of this work.

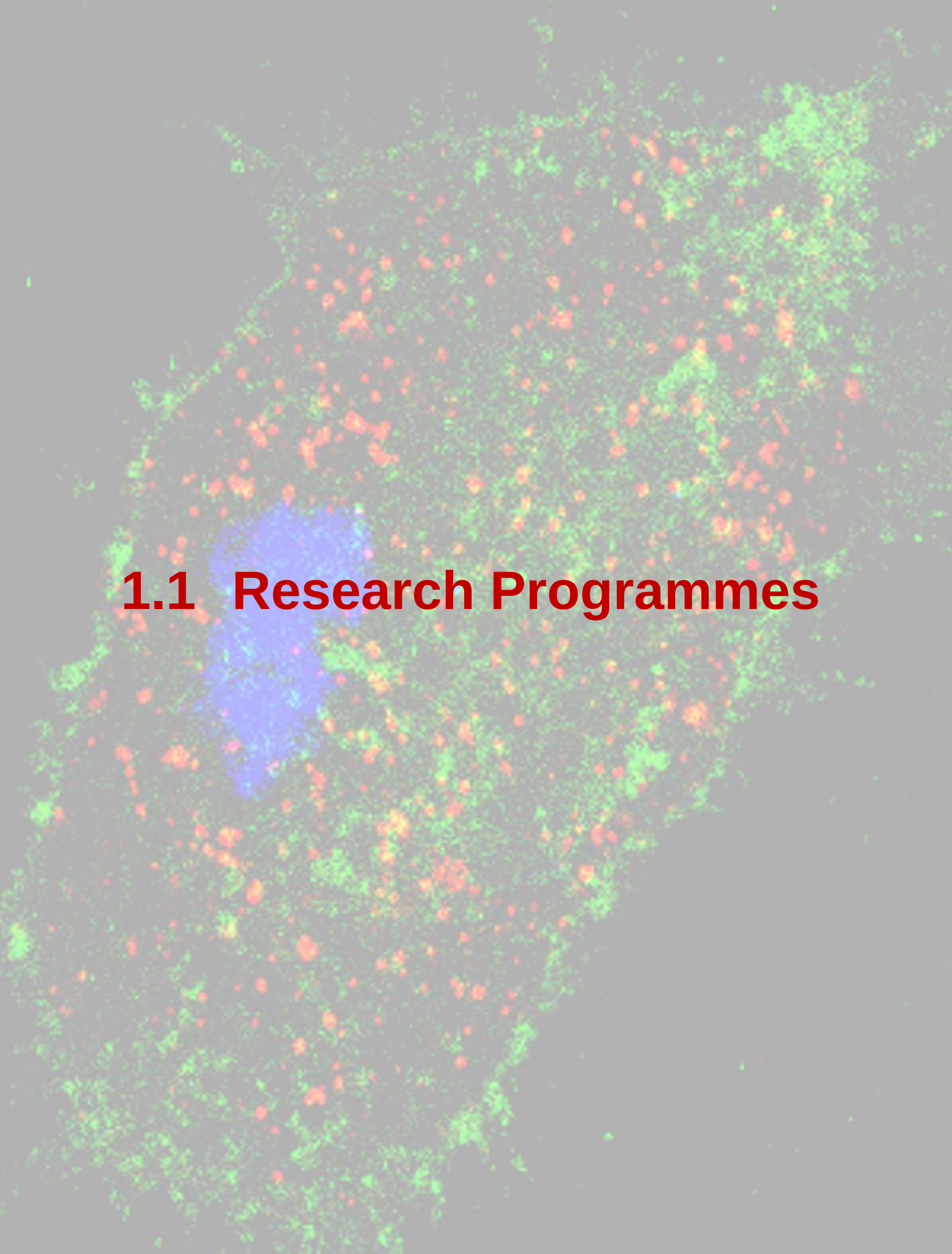
b) To carry out exploratory work in areas of biology with a view to aid the development of biochemical and biological technology in the country on a sound basis.

c) To train people in the advanced areas of biology to serve the needs of development in these areas, with special provision for short-term training of staff from other institutions in techniques for which adequate facilities may not exist elsewhere.

d) To provide centralized facilities in the country for new and modern techniques in the inter-disciplinary areas of biology, and to ensure that these facilities are so organized, maintained and administered that they can be put to maximal use by research workers from other laboratories and institutions in the country.

e) To interact adequately with other institutions doing basic or applied work in areas related to the activities of the Centre.

f) To collect, collate and disseminate information relevant to biological research.



1.1 Research Programmes

1.1 A
Research Summaries

AMIT ASTHANA

Applications of Microfluidics, Micro and Nanotechnology in Life Sciences

From left to right: Shahila Parween, Ira Bhatnagar, Amit Asthana, Naga Sowmya

RESEARCH INTERESTS:

- Non-conventional methods to fabricate microfluidic devices
- Point-of-care diagnostic devices based on paper-microfluidics
- Paper-based devices as Raman immune--sensors
- Biopolymer microfluidic devices for tissue engineering and cell culture
- Microdevices for 3D cell culture
- Generation of "site targeted" drug delivery vectors for drug delivery and diagnosis, using microfluidic devices

"The main aim of our group is to use micro and nanotechnology to address biological problems. The current research focus of the group centres-around developing affordable paper-based devices for clinical diagnostics".

Selected recent publications

- Shahila Parween, Debishree Subudhi P, Asthana A (2019). An affordable, rapid determination of total lipid profile using paper-based microfluidic device. *Sensors & Actuators: B. Chemical* 285: 405–412.

- Rayaprolu Anirudh, Srivastava SK, Bhati L, Anand K, Asthana A, Mohan Rao Ch (2019). Fabrication of cost-effective and efficient paper-based device for viscosity measurement. *Analytica Chimica Acta* 1044: 86-92.

- Ahsan SM, Thomas M, Reddy KK, Sooraparaju SG, Asthana A, Bhatnagar I (2018). Chitosan as biomaterial in drug delivery and tissue engineering. *International Journal of Biological Macromolecules* 110: 97-109.

- Bhatnagar I, Mahato K, Reddy KK, Ealla, Asthana A, Chandra P (2018). Chitosan stabilized gold nanoparticle mediated self-assembled gliPnanobiosensor for diagnosis of Invasive Aspergillosis. *International Journal of Biological Macromolecules* 110: 449-456.

- Pham UHT, Hanif M, Asthana A, Iqbal SM (2015). A microfluidic device approach to generate hollow alginate microfibers with controlled wall thickness and inner diameter. *Journal of Applied Physics* 117: 214703.

Patents Filed

- A novel facile aqueous based extraction of progesterone metabolites from faeces sample for non-invasive, simple, affordable and farmer friendly paper based kit for pregnancy detection in cattle and buffaloes, G. Umapathy, Amit Asthana, Chintalagiri Mohan Rao, Vinod Kumar, Gopi Suresh Oggu; Indian Patent, Application number 201911008655.

- A rapid, low cost process for the preparation of SERS substrate and SERS substrate prepared thereby, Amit Asthana, Mohan Rao Chintalagiri, Saurabh Kumar Srivastava, Gopi Suresh Oggu; Indian Patent, Application number 201811023895.

Highly sensitive, stable, uniform paper-based SERS substrates using *in-situ* synthesis of gold nanoparticles (with Dr.Ch Mohan Rao)

Surface-Enhanced Raman Spectroscopy (SERS) is a powerful surface-sensitive technique for molecular analysis. Its use is rather limited due to high cost, non-flexible rigid substrates such as silicon, alumina or glass and less reproducibility due to their non-uniform surfaces. Recently, paper-based SERS substrates, being of low cost and highly flexible alternatives, received a great deal of attention. We have developed a rapid, inexpensive method for the synthesis of gold nanoparticles (GNPs) on paper devices that could be utilized directly as SERS substrates. The GNPs have been prepared by reducing aurochloric acid with chitosan as a reducing as well as capping reagent, on a cellulose-based paper surface at 100ºC, undersaturated humidity conditions. The GNPs thus obtained were uniformly distributed on the surface and had a fairly uniform particle size with a diameter of 10±2nm. The substrate coverage of the resulting GNPs was directly dependent on the precursor's ratio, temperature and reaction time. Techniques such as TEM, SEM, and FE-SEM were utilized to determine the shape, size, and distribution of the GNPs on a paper substrate. The SERS substrate produced by this simple, rapid, reproducible and robust method showed exceptional performance and long-term stability, with a detection limit up to 1 pMconcentration of a test analyte, R6G. The present paper-based SERS substrates are cost-effective, reproducible and flexible and are suitable for field applications.

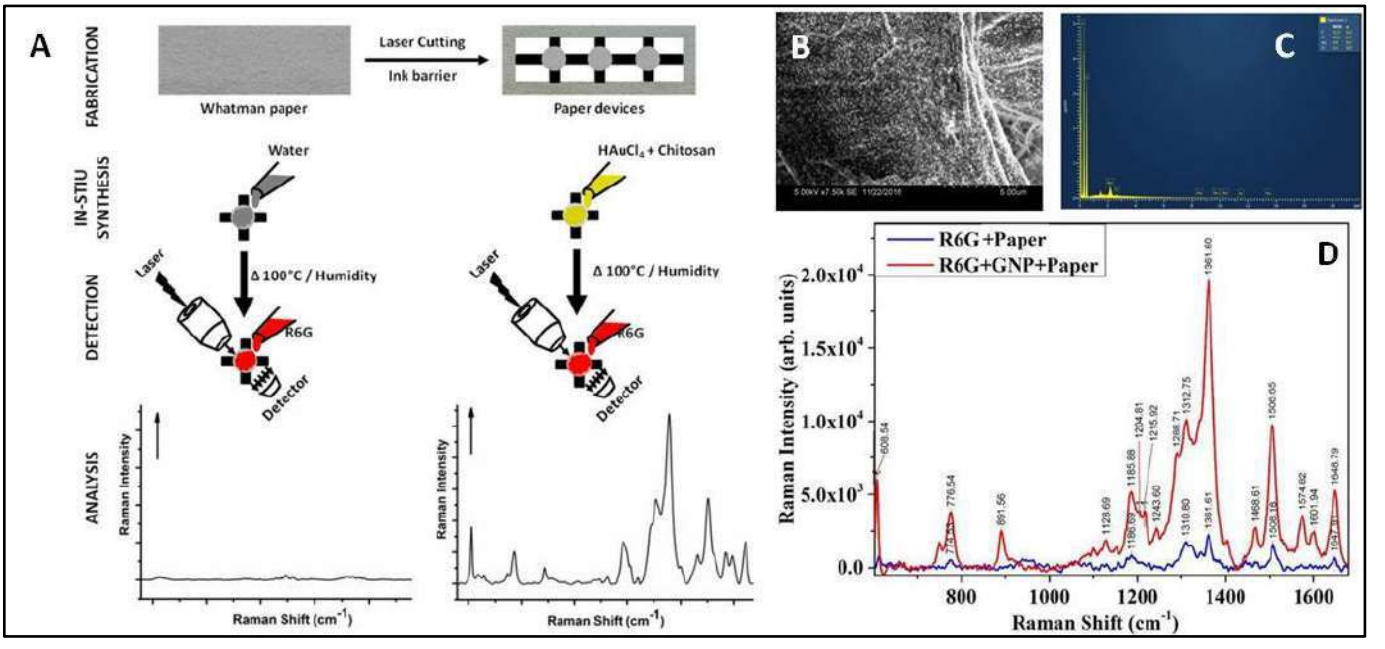


Fig. 1: (A) Schematic of process of fabricating paper-based SERS and its advantages **(B)** SEM image showing the as-synthesized GNP bound on paper-based devices at 100 °C reduction temperature and a reduction time of 60 mins with precursor mix of 25:15 :: chitosan : aurochloric acid, **(C)** shows the EDX analysis report with distinct elemental gold peak; **(D)** SERS spectra of 2.5 µL of 1 µMRhodamine 6G adsorbed on a paper substrate with and without the as-synthesized GNP's using a laser excitation wavelength of 633 nm.

Total lipid profile using paper-based microfluidic device

We have developed a novel surface modification method of the paper surface using 3-aminopropyltriethoxysilane (APTES) and gold nanoparticles. This functionalized paper (flower-shaped paper-based microfluidic devices (F-PMD) for simultaneous assay of multiple analytes) is used as a sensitive diagnostic sensor for determination of total lipid profiling (TLP) in a single device. We have determined total cholesterol along with low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides (TGL). The LDL and HDL were estimated by erasing the non-HDL and non-LDL components with a help of precipitating reagents. Standard graphs prepared by the present paper-based method nicely correlates with the conventional extimationmethods (calculated by Roche- COBAS C111 auto analyzer) with Pearson correlation coefficients (R) of 0.903, 0.9727, 0.9939 and 0.9866 0.9929, 0.9170 and 0.970 respectively for total cholesterol, LDL, HDL and triglycerides. The present assay device is intended to assist in the screening of individual's lipid level for diagnosis of disorders involving high cholesterol levels in the blood or for metabolic disorders associated with lipids and lipoproteins. As this is affordable, miniaturized, easily portable, self-testing and rapid, the present assay device can be used easily by pre-clinical, non-professionals, children in schools, and laboratories as a point-of-care diagnostic.

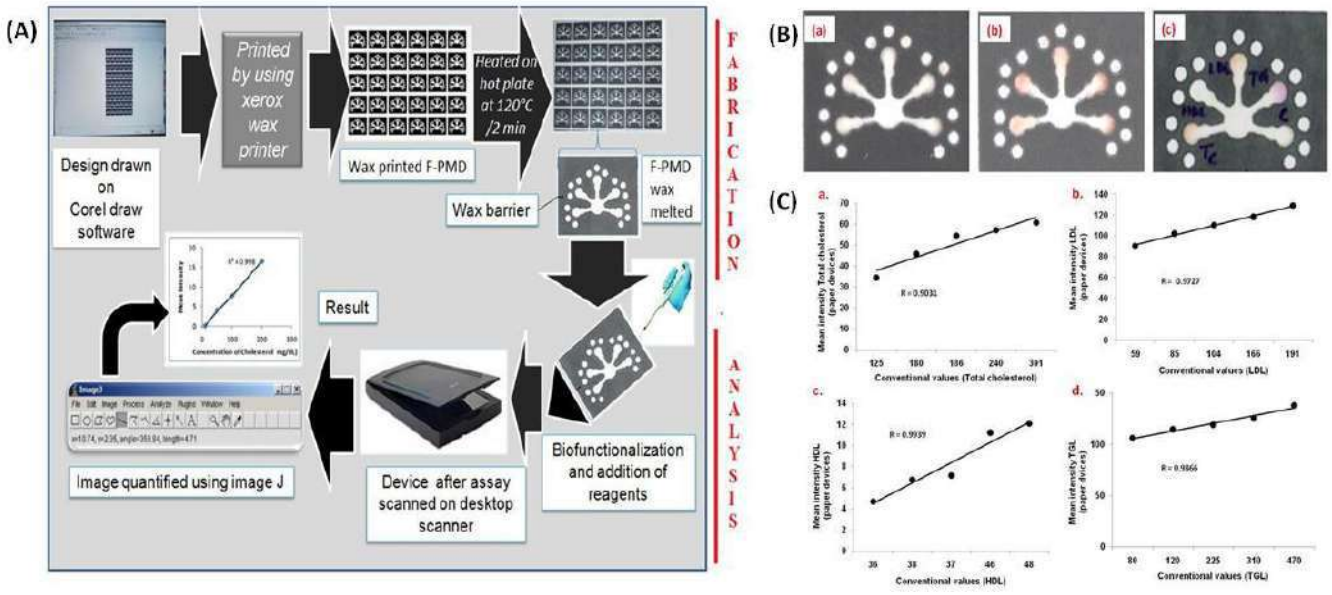


Fig 2: (A) Schematic representation of paper-based microfluidic device for total lipid profiling; (B) Total lipid profiling in F-PMD (a) Control, (b) Free cholesterol (no precipitating reagents added in any stem), (c) Total lipid detection; (C) Graph showing the correlation between the conventional values and values obtained from the present method for (A) Total cholesterol, (B) LDL (C) HDL, and (D) Triglycerides.

A S SREEDHAR

Stress Biology and Molecular Medicine

From left to right: Aakanksha Pant, Pankaj Kumar (Front Row)
A. Vijayalakshmi, K.R. Paithankar, A.S. Sreedhar, Shrikant P. Dharaskar, Akhil Kotwal (Back Row)

RESEARCH INTERESTS:

- Hsp90 in the epigenetic regulation of cancer
- Hsp90 in the cross-talk between acquired multidrug resistance and metastasis of cancer
- Trap1 in regulating mitochondrial dynamics and alternate energy metabolism in cancer

"Our group aims to understand the unconventional roles of cancer chaperone Hsp90 in tumor adaptations and thereby develop novel antitumor strategies to combat cancer."

Selected recent publications

- Kanugovi AV, Joseph C, Sreedhar AS (2016). The tumor suppressor $p16^{INK4a}$ expression bypasses 17AAG mediated cellular effects in human neuroblastoma, IMR-32. *Translational Medicine*. 6: 74.
- Kanugovi AV, Amere SS (2014). Oncogene de-addiction from Hsp90 induces senescence-like phenotype in malignant and metastatic tumor cells. *Molecular Biology of the Cell*. 25:3987.
- Sarangi U, Singh MK, Abhijnya KVV, Prasanna Anjaneya Reddy L, Siva Prasad B, Pitke VV, Paithankar KR, Sreedhar AS (2013). Hsp60 chaperonin acts as barrier to pharmacologically induced oxidative stress mediated apoptosis in tumor cells with differential stress response. *Drug Target Insights*. 7:35.
- Chaturvedi V, Ujwal Kumar J, Swamy CVB, Nandini R, Srinivas G, Kumaresan R, Singh, S and Sreedhar AS (2011). Repercussion of mitochondria structural deformity induced by anti-Hsp90 drug 17AAG in human tumor cells. *Drug Target Insights*. 5:11.

Heat shock proteins (Hsps) form the most ancient defense system in all living forms. Hsps are highly conserved and ubiquitously expressed proteins that play major roles in the maintenance of cellular homeostasis. Induced Hsps protect cells from various harmful stimuli. Increased Hsp expression is also found in several pathological conditions including cancer. We are interested in studying the unconventional roles of Hsps in tumor cells using molecular and chemotherapeutic approaches.

Hsp90 in the epigenetic regulation of cancer

Hsp90 is identified as a cancer chaperone involved in the conformational maturation and functional stabilization of mutated gene products, especially oncogenic kinases. In recent times Hsp90 inhibitors have emerged as novel anticancer agents. Cancer is an adaptive cellular response associated with chromatin remodeling and extensive epigenetic alterations. The role of Hsp90 in the epigenetic regulation of cancer is not explored. Earlier we reported that the regulation of the tumor suppressor retinoblastoma is under the control of Hsp90 *via* E2F family of transcription factors. However, Hsp90 activity and function is also known to be regulated by post-translational modifications such as phosphorylation, acetylation and nitrosylation. Acetylation of Hsp90 has been shown to decrease its interaction with client proteins. While HDAC6 in the cytoplasm is known to regulate Hsp90 activity, we discovered that HDAC3 in the nucleus regulates Hsp90 activity in tumor cells. The HDAC3-dependent Hsp90 regulation appeared to be important for helping tumor cells in tumor suppression. Pharmacological inhibition of Hsp90/HDAC3 leads to a significant decrease in nuclear HDAC3/Hsp90 levels respectively, suggesting an interdependent function between these two proteins. Further, HDAC3 also regulates the high affinity conformation of Hsp90 which is essential for its client protein interaction.

Hsp90 inhibition and multidrug resistance

Acquired multidrug resistance is also an adaptive cellular response suggested to be mediated by molecular chaperones. Earlier we reported that Hsp90 and P-glycoprotein (P-gp) expression correlates with multidrug resistant cancer phenotype. Further, we demonstrated that increased high affinity confirmation of Hsp90 is associated with enhanced drug efflux activity. Correlating with enhanced drug efflux activity, we also observed increased co-localization of Hsp90 and P-gp at the plasma membrane, more specifically at the cholesterol rich membrane microdomains called lipid rafts. Using *in silico* analysis and anisotropy measurements, we showed that cholesterol interacts with Hsp90. Unlike client proteins that prefer the highly charged hinge region of Hsp90 for their interaction, the C-terminal region that is enriched with CRAC motifs is found to be responsible for cholesterol interaction. Further, using tumor xenografts we show that drug resistant cells are metastatic and gain new homing properties. Subsequently, we found that these cells are sensitive to Hsp90 inhibition, but not to enhanced drug efflux activity. In contrast with the conventional understanding that drug resistant cancer cells are highly metastatic, and that these functions align in cancer cells, we demonstrate that they are independent. Using matrix metalloproteinase MMP7 over expressed drug resistant cancer cells, we show that drug resistance and metastasis are not linked. MMP7 over expressed cells show both enhanced drug resistance and metastasis, but interfering with Hsp90 functions or knocking down MMP7 specifically affected metastasis both *in vivo* and *in vitro*.

Trap1 in metabolic reprogramming and in the maintenance of mitochondrial integrity

Trap1 (TNF receptor associated protein 1) is a nuclear encoded, mitochondrial chaperone belonging to HSP90 super family. Earlier we showed that Trap1 expression increases with tumor aggression and is associated with enhanced mitochondrial fission, whereas its knockdown promotes mitochondrial fusion. Since Trap1 over expressing cells show enhanced ATP production independent of OXPHOS, we speculated that Trap-1-altered mitochondrial dynamics trigger alternate energy metabolism. Unlike Hsp90, Trap1 lacks the highly charged hinge region for the client protein interaction, and the C-terminal tetratricopeptide (TPR) motif that facilitates protein-protein interaction. For this reason, assigning a functional role of Trap1 in a particular molecular mechanism is rather difficult. Toward identifying Trap1 partners in mitochondria, tandem affinity purification

system (CTAP) was developed and used for MS/MS analysis. From the IDs obtained by pathway analysis we show that despite compromised OXPHOS, Trap1 is still associated with the intermediates of OXPHOS thus maintaining mitochondrial integrity in fission mitochondria. From the genomic data analysis, we found that Trap1 exhibits high copy number variation only in tumor cells thus drawing a correlation regarding why it may be associated with OXPHOS intermediates.

Our preliminary findings towards understanding the role of Trap1 in regulating mitochondrial dynamics using primary and non-tumor cells indicates that it interacts with MFF and DRP1 proteins that regulate mitochondrial fission. Our immunoprecipitation experiments were subsequently supported by co-immunofluorescence. We are now examining organelle specific fission protein interaction with Trap1. Since we observed altered mitochondrial dynamics affecting energy metabolism, we are working on how and when Trap1 shuts off OXPHOS and switches on altered energy metabolism. Considering that Trap1 promotes tumor growth, we examined tumor xenografts and found that Trap1 over expressing cells show new homing properties. Since conventional Hsp90 inhibitors failed to elicit organelle specific targeting, as reported earlier, we developed a nanaocarrier molecule that specifically targets mitochondria and selectively targets tumor cells. The *in vitro* effects of this nanocarrier were examined and it is now set for *in vivo* testing against tumor xenografts.

PURNIMA BHARGAVA

Epigenetic Mechanisms of Gene Regulation

Purnima Bhargava

RESEARCH INTERESTS:

- Transcription by yeast RNA polymerase III
- Epigenetic regulatory mechanisms
- Determinants of Nucleosome positioning

"We have shown that several chromatin modifiers and transcription factors of pol II associate with the transcription complex of pol III. We have also discovered that the Yeast Paf1 complex counters the replication stress on the pol III-transcribed genes, which are highly transcribed *in vivo*"

Selected recent publications

- Bhalla P, Shukla A, Vernekar DV, Arimbasseri AG, Sandhu KS, Bhargava P (2019). Yeast PAF1 complex counters the pol III accumulation and replication stress on the tRNA genes. *Scientific Reports* 9: 1-13.
- Bhalla P, Vernekar DV, Gilquin B, Coute Y, Bhargava P (2019). Interactome of the yeast RNA polymerase III transcription machinery constitutes several chromatin modifiers and regulators of the genes transcribed by RNA polymerase II. *Gene* 702: 205-214.
- Bhalla P, Vernekar DV, Shukla A, Gilquin B, Coute Y, Bhargava P (2018). Yeast PAF1 complex restricts the accumulation of RNA polymerase III and counters the replication stress on the transcribed genes. *BioRxiv* 339499.
- Shukla A, Bhargava P (2018). Regulation of tRNA gene transcription by the chromatin structure and nucleosome dynamics. *BBA-GRM* 1861: 295-309.
- Belagal P, Normand C, Shukla A, Wang R, Dez C, Bhargava P, Gadal O (2016). Decoding the principles underlying the frequency of association with nucleoli for RNA polymerase III-transcribed genes in budding yeast. *Molecular Biology of the Cell* E16-03-0145.

Compaction of the eukaryotic genome into chromatin restricts the access of transcription machinery to its templates. We have been trying to understand the mechanisms followed by the transcription machinery to overcome the repressive influence of the chromatin, using yeast as model eukaryotic system. In the budding yeast *Saccharomyces cerevisiae*, ~300 genes are found scattered on different chromosomes are transcribed by the enzyme RNA polymerase (pol) III. Pol III is dedicated to synthesizing the short, stable, non-coding RNAs, required for the vital cell processes like translation, ribogenesis and mRNA processing. Pol III is assisted by its two basal factors TFIIIC and TFIIIB, which assemble the transcription complex utilizing the intra-genic promoter elements. Several recent reports on regulation by chromatin-related mechanisms have unravelled novel mechanistic details which have a positive effect on transcription by pol III.

Our earlier measurement of pol III occupancy revealed that in the budding yeast, all target genes are actively transcribed but to different levels. Pol III transcription is precisely regulated under various stress conditions. In order to find new regulatory molecules, we used AP-MS/MS approach for identifying the complete repertoire of interacting partners of the yeast pol III transcription machinery. A large number of proteins involved in chromatin biology, ribogenesis, stress signaling as well as pol II transcription were identified.

Last year, we reported the interaction of the Paf1 complex, one of the well-studied transcription factors of pol II, with the pol III transcription complex. We found that Paf1 is present at different levels on the different pol III-transcribed genes and its deletion results in gene-specific increase of pol III transcription. A high transcription rate of pol III-transcribed genes *in vivo* causes replication fork stalling at the genes, making them prone to DNA damage. Paf1 deletion or exposure of the cells to the genotoxin HU causes slow growth. We found that Paf1 plays a protective role by reducing the transcription activity and pol III levels on the genes. Pol III occupancy at the genes is reduced when exposed to HU but highly increased when Paf1 is deleted. We observed that in normally cycling cells, Paf1 is involved in regulating the release of pol III from its pause in a regulated manner. The gene-specific occupancies and effects of Paf1 could be related to possible inclusion of the target genes in different, independently acting chromosome domains. Therefore, we looked for proximity of tRNA genes and Paf1-binding sites to replication origins and TAD (Topologically associated domain) boundaries, which were reported to mark the transition between early and late replicating genomic regions in mouse and human cells. Our analyses of the existing data in the public domain found gene-specific differences in proximity of the tRNA genes to replication origins and TAD boundaries. The tRNA genes exhibit significant enrichment around the replication origins (p-value=0.0004) and the TAD boundaries (p-value=0.02) when compared to the ORFs in the yeast genome. In comparison, Paf1 exhibited highly significant enrichment around the TAD boundaries (p=4.8E-46) but not replication origins. This indicates that replication fork stalling and DNA damage are not spatial location-dependent properties of the tRNA genes and gene-specific behaviour may be a culmination of various parameters like transcription activity, spatial location, association with different gene clusters and topological features of the genome, as evidenced by our earlier studies.

Apart from Paf1, we found the FACT complex also to interact with the pol III transcription complex. This complex is required for transcription elongation through the nucleoosmes on pol II-transcribed genes. It travels with pol II on the genes, and as a chaperone, participates in the exchange of histone dimers H2A/H2B. Last year, we had reported high levels of FACT subunit Spt16 at the 3'-end of the tRNA genes, where Spt16 maintains the H2A.Z levels. However, both H2A.Z and Spt16 were not required for tDNA transcription by pol III. Instead, Spt16 traveling with pol III could serve tolink H2A.Z levels with the DS nucleosome dynamics, which plays a regulatory role in differential transcription of isogenes. Therefore, by associating with pol III, Spt16 has a direct approach to sense the transcription status at the pol III-transcribed genes. In order to gain further insight in the H2A.Z-mediated stress-sensing mechanisms at the tRNA genes, we have performed several genome-wide mapping experiments. The acquired data is currently under analysis.

VENKAT CHALAMCHARLA

Transcription and Chromatin Regulation

From left to right: Anubhav Bhardwaj, Venkat Chalamcharla, Annapoorna

RESEARCH INTERESTS:

- Transcriptional control of gene expression
- Epigenetics and chromatin organization
- Maintenance of genome stability

"Our lab is interested in uncovering the rules that govern gene regulation. We focus on molecular mechanisms involved in transcriptional elongation control by RNA Polymerase II, which dictategene expression patterns duringdevelopment and disease".

Selected recent publications

- H. Diego Folco, Venkata R. Chalamcharla, Tomoyasu Sugiyama, Gobi Thillainadesan, Martin Zofall, Vanivilasini Balachandran, Jothy Dhakshnamoorthy, Takeshi Mizuguchi and Shiv I. S. Grewal (2017). Untimely expression of gametogenic genes in vegetative cells causes uniparental disomy. *Nature* 543: 126-130.
- Tomoyasu Sugiyama, Gobi Thillainadesan, Venkata R. Chalamcharla, Zhaojing Meng, Vanivilasini Balachandran, Ming Zhou and Shiv I.S. Grewal (2016). *Enhancer of rudimentary* cooperates with Mmi1 and conserved RNA processing factors to promote meiotic mRNA decay and facultative heterochromatin assembly. *Molecular Cell* 61: 747-759.
- Venkata R. Chalamcharla, H. Diego Folco, Jothy Dhakshnamoorthy, and Shiv I. S. Grewal (2015). A conserved factor Dhp1/Rat1/Xrn2 triggers premature transcription termination and nucleates heterochromatin to promote gene silencing. *Proceedings of the National Academy of Sciences, USA* 112: 15548-15555.
- Nathan N. Lee, Venkata R. Chalamcharla, Francisca Reyes-Tercu, Sameet Mehta, Martin Zofall, Vanivilasini Balachandran, Jothy Dhakshnamoorthy, Nitika Taneja, Soichiro Yamanaka, Ming Zhou, Shiv I.S. Grewal(2013).

Mtr4-like protein coordinates nuclear RNA processing for heterochromatin assembly and for telomere maintenance. *Cell* 155: 1061-1074.

- Venkata R. Chalamcharla, Joan M. Curcio, Marlene Belfort (2010). Nuclear expression of a group II intron is consistent with spliceosomal intron ancestry*" Genes & Development* 24: 827-836.

Our interests lie in the areas of gene regulation, epigenetics and genome stability. We work on molecular mechanisms that control transcriptional elongation by RNA Polymerase II and its impact on chromatin and DNA damage response. We use the simple model organism *Schizosaccharomyces pombe* (fission yeast) as well as mammalian cells as our experimental systems.

We study a phenomenon called Promoter-proximal Po III pausing, which is a major regulatory check point for spatial and temporal control of gene expression in eukaryotes, particularly in metazoans. Po III pausing near promoters is also observed in fission yeast *S. pombe*, but its mechanism, regulation and function are unknown. A major goal of our lab istoidentify the key genetic and epigenetic factors that control the stability of 5' paused Pol II complexes, and understand how they affect transcriptional dynamics.

We are also investigating the transcriptional control mechanisms involved in the maintenance and regulation of the ubiquitous but poorly understood cellular state called quiescence (also called the G_0 phase of the cell cycle). Recent studies found surprisingly active transcription at several gene *loci* in the quiescent state. Moreover, many key genes required for cells to re-enter the cell division cycle are considered to be in a poised transcriptional state. We hope to understand how gene transcription patterns are maintained in quiescent cells, and how they can be rewired to prioritize cell growth during the quiescence exit (Fig. 1).

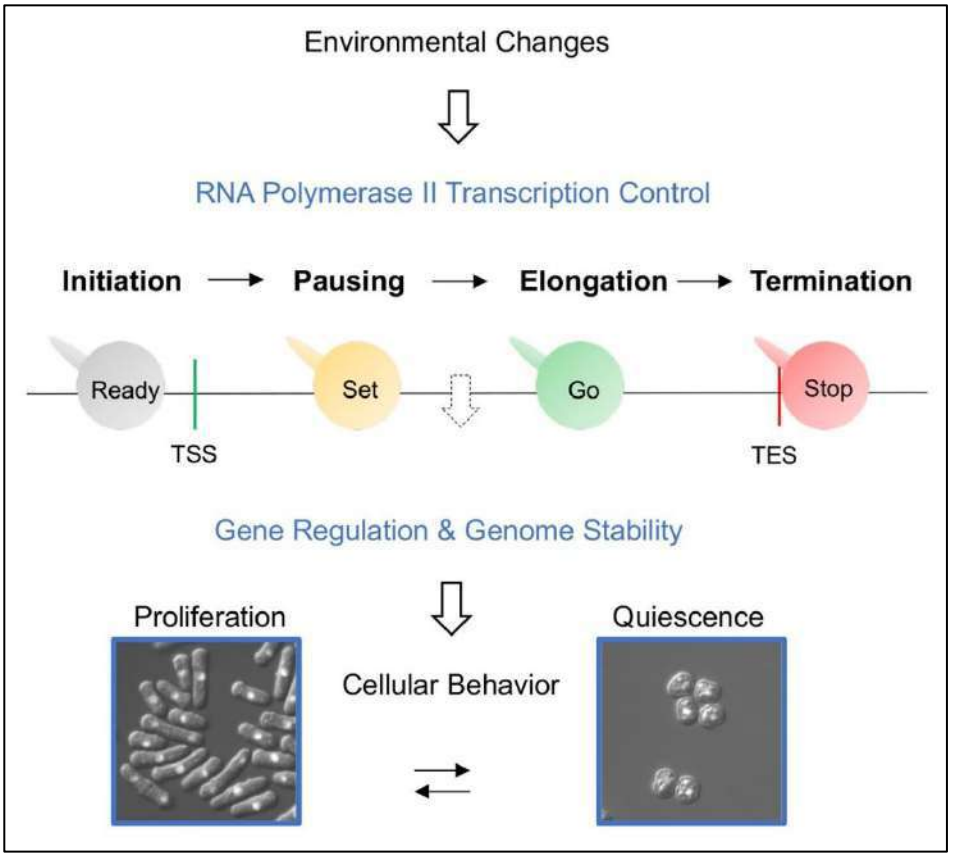


Fig. 1: Schematic of our lab's research focus

GIRIRAJ RATAN CHANDAK

Genomic Research on Complex Diseases

From left to right (starting from last row): Ashutosh Singh Tomar, Akshay Dedaniya, Ajay Deepak Verma, Shoma Kumaresh Naskar, Alagu Sankareswaran, Swati Bayyana, Lovejeet Kaur, Prachand Issarapu, Sara Sajjadi, P S K D B Punya Sri, Inder deo Mali, Neha Dhiman, G R Chandak, Seema Bhaskar, Jyothi V, Mounika Ch, Ashok P, Manisha Arumalla, Vinay D, Shagufta Tasneem, Mobeen Shaikh, Radhika P Ramachandran

RESEARCH INTERESTS:

- Gene-nutrient interaction and Developmental Origin of Health and Diseases (DOHaD)
- Genetic susceptibility of type 2 diabetes mellitus and related intermediate traits
- Pre- and peri-conceptional nutritional intervention to understand causality in DOHaD

"Our group has provided evidence that genetic basis of complex diseases and related intermediate traits in Indians have unique features. He has also identified that vitamin B12 status epigenetically regulates genes involved in key pathways implicated in type 2 diabetes and related intermediate traits including obesity as well as a unique microRNA that influences one-carbon metabolism. Further molecular studies on pre- and peri-conceptional nutritional intervention cohorts have identified differential methylation of critical genes that also show characteristic of metastable epialleles suggesting crucial role of early influence of maternal nutrition in development."

Selected recent publications

- Lasher D, Szabó A, Masamune A, Chen JM, Xiao X, Whitcomb DC, Barmada MM, Ewers M, Ruffert C, Paliwal S, Issarapu P, Bhaskar S, Mani KR, Chandak GR, Laumen H, Masson E, Kume K, Hamada S, Nakano E, Seltsam K, Bugert P, Müller T, Groneberg DA, Shimosegawa T, Rosendahl J, Férec C, Lowe ME, Witt H, Sahin-Tóth M (2019). Protease-Sensitive Pancreatic Lipase Variants Are Associated With Early Onset Chronic Pancreatitis. *American Journal of Gastroenterology* 114: 974-983.
- Dhillon BK, Chopra G, Jamwal M, Chandak GR, Duseja A, Malhotra P, Chawla YK, Garewal G, Das R (2018). Adult onset hereditary hemochromatosis is associated with a novel recurrent Hemojuvelin (HJV) gene mutation in north Indians. *Blood Cells Molecular Diseases* 73: 14-21.
- James P, Sajjadi S, Tomar AS, Saffari A, Fall CHD, Prentice AM, Shrestha S, Issarapu P, Yadav DK, Kaur L, Lillycrop K, Silver M, Chandak GR; EMPHASIS study group (2018). Candidate genes linking maternal nutrient

exposure to offspring health via DNA methylation: a review of existing evidence in humans with specific focus on one-carbon metabolism. *International Journal of Epidemiology* 47: 1910-1937.

- Yadav DK, Shrestha S, Chandak GR (2018). Identification and Characterization of cis-Regulatory Elements "Insulator and Repressor" in PPARD Gene. *Epigenomics* 10: 613-627.

Patents filed

- Giriraj Ratan Chandak, Dr. Sumit Paliwal, Ms Swati Bayyana, Mr Vinay Donnipadi (2019). A method for detection of Genetic Disorders.

The research focus of my group has been to understand the role of individual genes and dissect gene-gene and gene-environment, especially gene-nutrient interaction in the etiopathogenesis of common complex diseases such as diabetes mellitus and metabolic syndrome. This is based on the recurrent observation that the phenotype and the clinical course of many complex diseases in Indians is different as compared to Europeans. We have also provided evidence for fetal programming of birth weight and future cardio-metabolic risk as a result of intrauterine exposure, especially maternal B12.

A genetic risk score improves diagnosis of type 1 diabetes in Indian patients

Type 1 diabetes (T1D) is a significant problem in South Asians but there is little research focussing on the genetic associations. Misclassification of T1D and type 2 diabetes (T2D) could be a problem in young adults due to high prevalence of early onset T2D in this population at lower BMI. We have previously shown that a T1D genetic risk score (GRS) can be used to discriminate T1D from T2D in Europeans. We tested the ability of the T1D GRS to discriminate T1D from T2D and controls in Indians with diabetes.

We studied Indian subjects from Pune, India; T1D (n=305), T2D (n=352) and controls (n=334). We genotyped 9 SNPs capturing common HLA risk (DR3 and DR4) and non-HLA risk for T1D in Europeans. A 9-SNP T1D GRS was calculated and its ability to discriminate T1D from T2D and control subjects was assessed. We compared the results with Europeans from Wellcome Trust Case Control Consortium study of T1D (n=238), T2D (n=1914) and controls (n=2938).The T1D GRS was discriminative of T1D and T2D (receiver operator characteristic area under the curve [ROC AUC (95% CI) = 0.81 (0.77–0.83)] but had lower power compared to Europeans [ROC AUC (95% CI) = 0.87 (0.86-0.88), P<0.0001]. The HLA SNPs contributed to the majority of discriminative power [ROC AUC (95% CI) = 0.76 (0.72-0.79) for DR3/DR4 GRS].

Thus, a T1D GRS using 9 SNPs defined in Europeans is discriminative of T1D in Indians and may be useful for classifying diabetes in Indians.

Effect of pre- and peri-conceptional maternal micronutrient intervention on children's DNA methylation

Micronutrient deficiency in pregnant women is associated with low birth weight, poor cognitive functions and health problems in their offspring. It is well established that their effects penetrate even into adulthood, manifesting through an increased vulnerability to non-communicable chronic diseases such as type 2 diabetes, cardiovascular diseases, obesity etc. Long-term effects of early-life nutritional exposures require mechanisms through which 'memory' of an individual's *in-utero* nutritional environment is retained and influences health outcomes in later life. DNA methylation, which can regulate orchestrated expression of several genes critical during fetal development, is a potential mechanism. Earlier studies in humans have linked altered methylation loci to micronutrient deficiency during pregnancy. However, there are no studies till date that have examined the effect of maternal peri-conceptional micronutrient interventions on children's DNA methylation in a randomized control trial setting.

We carried out EMPHASIS (**E**pigenetic **M**echanisms linking **P**re-conceptional nutrition and **H**ealth **AS**sessed in **I**ndia and sub-**S**aharan Africa, ISRCTN14266771) study, which is a follow up of two randomized control trials designed to investigate the epigenetic mechanisms through which maternal nutrition during pregnancy influences offspring health. Mumbai Maternal Nutrition Project, India

(MMNP; n=698) and the Peri-conceptional Maternal Micronutrient Supplementation Trial, The Gambia (PMMST; n=293) make up the intervention cohorts for EMPHASIS study. In the MMNP cohort, the intervention was a daily snack made from locally available micronutrient rich foods supplemented before and throughout pregnancy, while PMMST was a UNIMAP tablet supplemented daily to pre-pregnant women until the identification of pregnancy. We have earlier shown that the supplementation in the Indian cohort led to increase in offspring birthweight while in the Gambian cohort, season of conception plays a major role in various child outcomes. These findings add further support to the objectives of EMPHASIS study.

We conducted independent Epigenome-wide association studies (EWAS) on children (aged 5-9 yrs) in these cohorts using the methylation data generated on Infinium Methylation EPIC Array, which provides methylation status of over 850,000 CpG sites across the whole genome. EWAS in the Indian cohort identified a single differentially methylated position (DMP) that mapped to transmembrane protein 106A (*TMEM106A*) gene passing false discovery rate (FDR) 5% threshold, however, magnitude of the effect was very small (effect size<0.1%). In a region-based analysis, a statistically significant differentially methylation region (DMR; adjusted P-value=$5.7x10^{-18}$) mapping to Paternally Expressed Gene 10 (*PEG10*) was found to be associated with the intervention. In the Gambian cohort, six DMPs passed the pre-specified FDR threshold. Interestingly, the top four DMPs mapped to a single gene named Endothelial Cell Specific Molecule-1 (*ESM1*) with effect sizes of -2 to -5%. The remaining two DMPs mapped to Catenin Alpha 2 (*CTNNA2*) and Cadherin 18 (*CDH18*). DMR analysis showed *ESM1* and Leucine Zipper Tumour Suppressor gene1 (*LZTS1)* to be differentially methylated at the region level. Bioinformatic analysis showed that *ESM1* DMR extends over the transcription start site (TSS), 5' UTR and first exon of the most highly expressed transcript with enriched histone regulatory marks. The *LZTS1* DMR mapped to an intronic region, ~2Kb from the TSS and showed reduced chromatin accessibility as evidenced by the absence of DNase hypersensitive sites and the absence of TF binding indicative of a repressor role for this region. A significant correlation *(p*<$1.1x10^{-5}$) between blood and buccal tissues as representative of two different germ layers of origin indicates that the methylation of these nutritionally sensitive regions was laid during early stages of embryonic development hence could be plausible metastable epi-alleles. Technical validation of these results using pyrosequencing confirmed the initial observations.

Further, we used the high throughput genotype data generated on same samples using Illumina Global Screening Array to investigate the genetic influence on the significant CpGs and found that cis SNP rs1423249 is a strong methylation Quantitative Trait Locus (mQTL) for the *ESM1* CpGs. However, the effect of intervention and mQTL was independently significant and similar in magnitude. Hence, we speculated that methylation of *ESM1* CpGs is regulated independently by nutritional intervention and by mQTL SNP present in the 3'UTR of the gene. Further experiments are in progress to elucidate the downstream effects of methylation to these nutrition-sensitive DMRs.

Identifying *ESM1*, as a nutrition-sensitive locus is an important finding since earlier studies have associated it with both maternal and neonatal factors including gestational diabetes, pre-eclampsia and birthweight owing to its role in early stages of development. We are now examining the associations between DNA methylation and phenotypic outcomes in the children which will provide better mechanistic understanding of these putative links.

Diet induced paternal vitamin B_{12} deficiency shows sex specific pattern of programming in *Wistar* rat offspring

Parental nutritional deficiencies are known to influence life trajectories of their offspring. Previously, using Wistar rat model, we demonstrated that severe maternal vitamin B12 (B12) deficiency (~70%) could significantly affect their pups' cardiometabolic health

as evidenced by altered glucose and lipid metabolism. Recently we showed that mild deficiency of maternal B12 (~27%) led to programming of F1 male pups only who showed high triglycerides and low HDL-cholesterol levels providing a clue towards probable sexual dimorphic burden of cardiometabolic events through B12 deficiency. Thus, to understand whether paternal B12 deficiency also has any trans-generational and intergenerational effects, diet induced Wistar rat model of paternal B12 deficiency was generated (Fig 1A). We observed low plasma B12 and high homocysteine levels (Fig 1B) and higher bodyweight and fat mass in them (Fig 1C). To assess the intergenerational effect of paternal B12 deficiency, both deficient and control male rats were mated with control females (Fig 1D). F1 pups born to B12 restricted (VR) fathers were low birthweight and specifically female pups had increased fat mass (Fig. 4E & F).

The results highlight the criss-cross pattern of effects of parental B12 deficiency to their offspring. Presently, we are generating F2 animals to investigate the transgenerational inheritance and further intend to understand the molecular signatures responsible for the sexual dimorphic effects in both maternal and paternal B12 deficiency models.

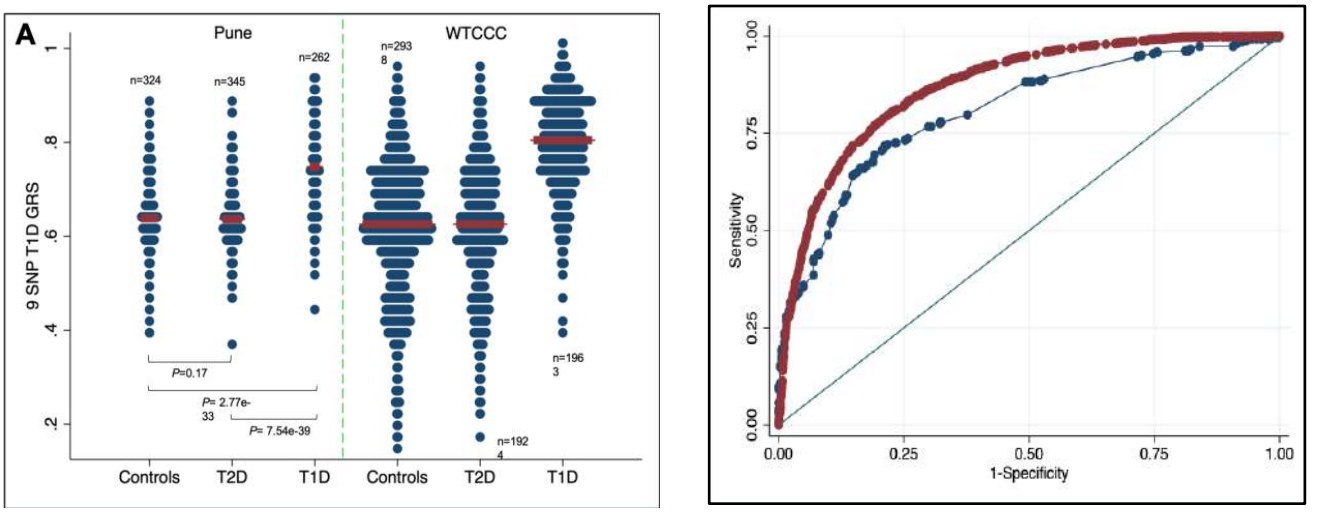


Fig. 1: ***Comparison of the ability of a 9 SNP GRS to discriminate between T1D and T2D in Indians from the WELLGEN study and individuals of European descent from the WTCCC study.*** A: Dot plot of 9 SNP T1D GRS in T1D, T2D and controls in Indians and Europeans in WTCCC. The width of the blue bars indicates frequency, and the red line is the median B: ROC curves showing the power of the 9 SNP T1D GRS to discriminate individuals with T1D from those with T2D in Indians (blue: AUC (95% CI) = 0.81 (0.77-0.83)] and Europeans in WTCCC (red: AUC (95% CI) = 0.87 (0.86-0.88)]. SNP, single nucleotide polymorphism; T1D, type 1 diabetes; T2D, type 2 diabetes; WELLGEN, Wellcome Genetic Study; WTCCC, Wellcome Trust Case Control Consortium; ROC, Receiver operating characteristics; GRS, genetic risk score; AUC, area under curve.

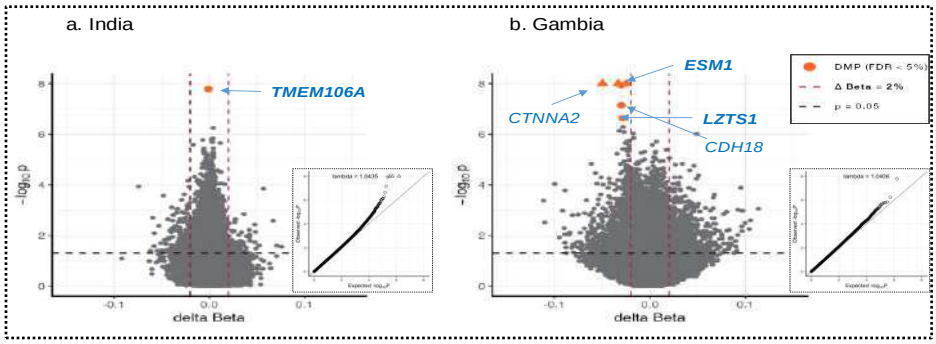


Fig. 2: Volcano plots for each cohort showing a difference in methylation Beta values between intervention and control for all CpGs tested. The dashed vertical and horizontal lines indicate thresholds for delta Beta 2% and nominal p=0.05 respectively. Coloured points show DMPs - significant CpGs with FDR< 5%, a triangle indicates a point falling outside the plot area. Inset figures show Quantile-Quantile plots for the respective cohorts. DMP, differentially methylated position; FDR, false detection rate.

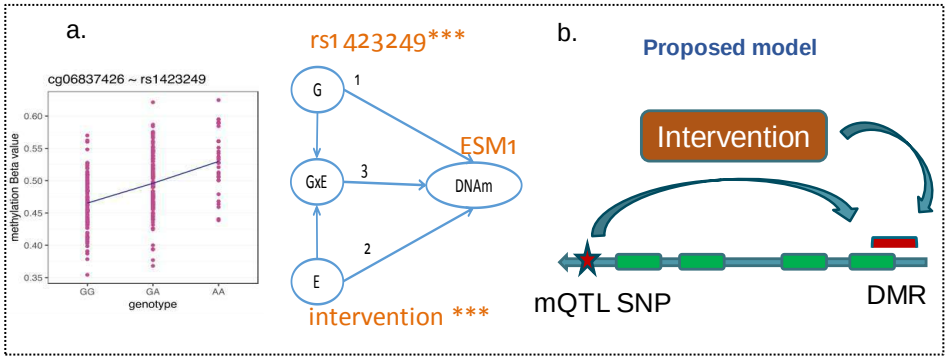


Fig. 3: Genetic influence on nutrition-sensitive loci (mQTL Analysis): a) Plots showing the effect of mQTL rs1423249 on methylation status at an ESM1 CpG. b) Schematic representation of the proposed model for the regulation of DNA methylation on nutrition-sensitive ESM1 DMR. mQTL, *methylation quantitative trait locus; DMR, differentially methylated region, ESM1, endothelial specific molecule 1.*

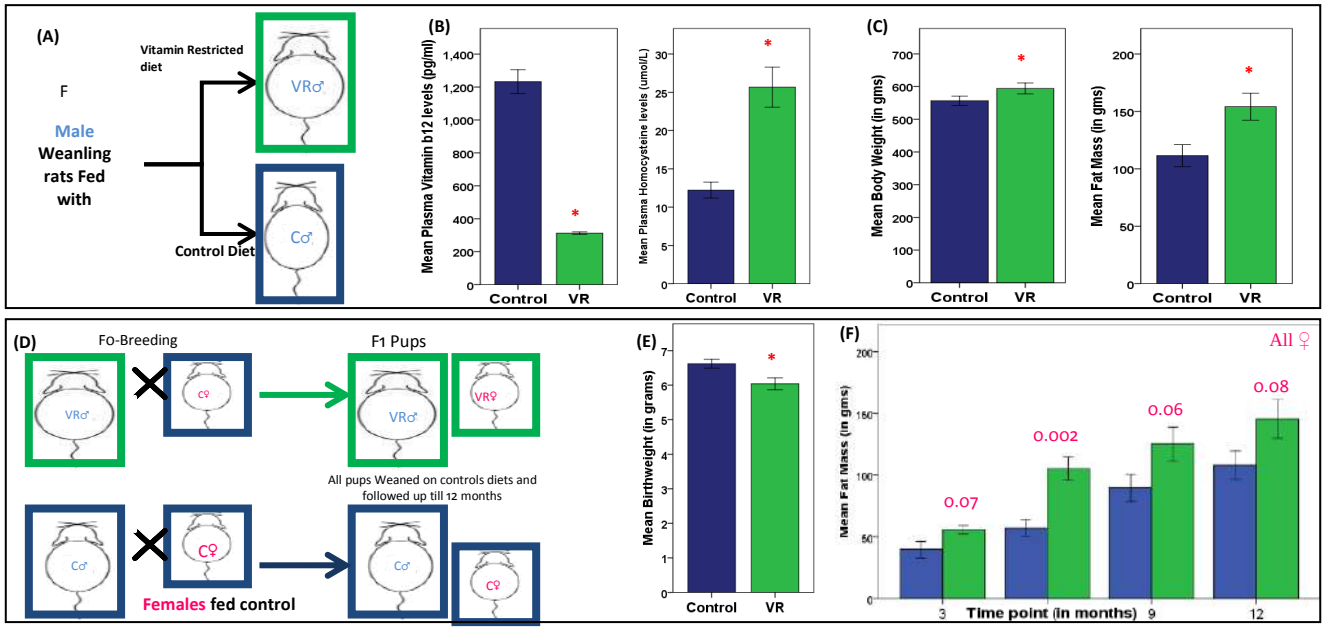


Fig. 4: Generation of paternal Vitamin B12 Restricted *Wistar* rat model. (A) Protocol; (B) Plasma B12 and homocysteine elevels; (C) Body weights and fat mass at 12 months age. (D) Experimental procedure for generating F1. (E) Birth weights of F1 Pups. (F) Trend of fat mass in F1 female pups. (Data presented as Mean±SEM). VR, vitamin B12 restricted; C, controls.

AMITABHA CHATTOPADHYAY

Membrane and Receptor Biology

From left to right: Parijat Sarkar, Sarosh N. Fatakia, G. Aditya Kumar, Subhashree S. Sahu, Md. Jafurulla, Bhagyashree D. Rao, Ashwani Sharma, Amitabha Chattopadhyay, Abhishek Kumar, Sreetama Pal, Sandeep Srivastava, K. Venkatalaxmi

RESEARCH INTERESTS:

- Interaction of membrane lipids and cytoskeletal proteins with G protein-coupled receptors: implications in health and disease
- Membrane cholesterol in membrane protein structure and function
- Endocytosis and intracellular regulation of G protein-coupled receptors
- Role of host cell membranes in the entry of pathogens
- Dynamics of solvent relaxation in membranes and proteins
- Novel applications of membrane dipole potential in membrane biology

"Our major focus is on a comprehensive understanding of the subtle interplay between G protein-coupled receptors (GPCRs) and membrane lipids with far reaching implications in health and disease, utilizing a judicious combination of biophysical, biochemical, cell biological and computational approaches"

Selected recent publications

- Kumar GA, Sarkar P, Jafurulla M, Singh SP, Srinivas G, Pande G, Chattopadhyay A (2019). Exploring endocytosis and intracellular trafficking of the human serotonin$_{1A}$ receptor. *Biochemistry* 58: 2628-2641 (*featured on the cover*).
- Fatakia S, Sarkar P, Chattopadhyay A (2019). A Collage of Cholesterol Interaction Motifs in the Serotonin$_{1A}$ Receptor: An Evolutionary Implication for Differential Cholesterol Interaction. *Chemistry and Physics of Lipids* 221: 184-192.
- Rao BD, Shrivastava S, Pal S, Chattopadhyay A (2019). Effect of Local Anesthetics on the Organization and Dynamics of Hippocampal Membranes: A Fluorescence Approach. *The Journal of Physical Chemistry B* 123: 639-647.
- Pal S, Samanta N, Das Mahanta D, Mitra RK, Chattopadhyay A (2018). Effect of Phospholipid Headgroup Charge on the Structure and Dynamics of Water at the Membrane Interface: A Terahertz Spectroscopic Study. *The Journal of Physical Chemistry B* 122: 5066-5074 (*featured on the cover)*.

- Sengupta D, Prasanna X, Mohole M, Chattopadhyay A (2019). Exploring GPCR-lipid Interactions by Molecular Dynamics Simulations: Excitements, Challenges and the Way Forward. *The Journal of Physical Chemistry B* 122: 5727-5737 (*featured on the cover*).

Endocytosis and intracellular trafficking of the human serotonin$_{1A}$ receptor

G protein-coupled receptors (GPCRs) represent the largest class of receptors involved in signal transduction across cell membranes and are major drug targets in all clinical areas. Endocytosis of GPCRs offers a regulatory mechanism for sustaining their signaling within a stringent spatiotemporal regime. In this work, we explored agonist-induced endocytosis of the human serotonin$_{1A}$ receptor stably expressed in HEK-293 cells and the cellular machinery involved in receptor internalization and intracellular trafficking. The serotonin$_{1A}$ receptor is a popular GPCR implicated in neuropsychiatric disorders such as anxiety and depression and serves as an important drug target. In spite of its pharmacological relevance, its mechanism of endocytosis and intracellular trafficking is less understood. In this context, we have utilized a combination of robust population-based flow cytometric analysis and confocal microscopic imaging to address the path and fate of the serotonin$_{1A}$ receptor during endocytosis. Our results, utilizing inhibitors of specific endocytosis pathways and intracellular markers, show that the serotonin$_{1A}$ receptor undergoes endocytosis predominantly via the clathrin-mediated pathway and subsequently recycles to the plasma membrane via recycling endosomes. These results would enhance our understanding of molecular mechanisms of GPCR endocytosis and could offer novel insight into the underlying mechanism of antidepressants that act via the serotonergic pathway. In addition, our results could be relevant in understanding cell (or tissue)-specific GPCR endocytosis.

Local anesthetics in hippocampal membrane organization and dynamics

Understanding the mechanism of action of local anesthetics has been challenging. We previously showed that the local anesthetic phenylethanol (PEtOH) inhibits the function of serotonin$_{1A}$ receptor, which is a member of the G protein-coupled receptor family and a neurotransmitter receptor. With the objective of gaining insight into the molecular mechanism underlying the anesthetic (PEtOH) action, we monitored the organization and dynamics of hippocampal membranes using multiple fluorescent reporters, which include a molecular rotor (BODIPY-C12) and a voltagesensitive probe (4-(2-(6-(dioctylamino)-2-naphthalenyl)- ethenyl)-1-(3-sulfopropyl)-pyridinium inner salt) (di-8-ANEPPS), besides pyrene. These interfacial membrane probes were chosen because membrane partitioning of PEtOH would be reflected in the membrane interfacial environment. Taken together, we report a reduction in dipole potential and microviscosity of hippocampal membranes, with a concomitant increase in lateral diffusion in the presence of PEtOH. The reduction in membrane dipole potential induced by PEtOH constitutes one of the first experimental demonstrations on the modulation of membrane dipole potential by local anesthetics. Our results assume significance in view of previous reports that correlate membrane-perturbing effects of local anesthetics to their anesthetic action. We envision that insights into the interaction of local anesthetics with membranes could serve as a crucial link in developing a comprehensive understanding of the molecular mechanisms involved in anesthesia.

Effect of phospholipid head group charge on the structure and dynamics of water at the membrane interface: a terahertz spectroscopic study

Biological membranes are highly organized supramolecular assemblies of lipids and proteins. The membrane interface separates the outer (bulk) aqueous phase from the hydrophobic membrane interior. In this work, we have explored the microstructure and collective dynamics of the membrane interfacial hydration shell in zwitterionic

and negatively charged phospholipid membrane bilayers using terahertz time-domain spectroscopy. We show here that the relaxation time constants of the water hydrogen bond network exhibit a unique “rise and dip” pattern with increasing lipid concentration. More importantly, we observed a dependence of the critical lipid concentration corresponding to the inflection point on the charge of the lipid headgroup, thereby implicating membrane electrostatics as a major factor in the microstructure and dynamics of water at the membrane interface. These results constitute one of the first experimental evidences of the modulation of the dielectric relaxation response of membrane interfacial water by membrane lipid composition in a concentration-dependent manner. Lipid-stringent membrane hydration could be relevant in the broader context of lipid diversity observed in biological membranes and the role of negatively charged lipids in membrane protein structure and function.

Cholesterol interaction motifs in the serotonin$_{1A}$ receptor: evolutionary implication

The serotonin$_{1A}$ receptor is a representative member of the G protein-coupled receptor (GPCR) superfamily and acts as an important drug target. In our previous work, we comprehensively demonstrated that membrane cholesterol is necessary in the organization, dynamics and function of the serotonin$_{1A}$ receptor. In this context, analysis of high-resolution GPCR crystal structures in general and in silico studies of the serotonin$_{1A}$ receptor in particular, have suggested the presence of cholesterol interaction sites (hotspots) in various regions of the receptor. In this work, we have identified an evolutionarily conserved collage of four categories of cholesterol interaction motifs associated with transmembrane helix V and the adjacent intracellular loop 3 fragment of the vertebrate serotonin$_{1A}$ receptor. This collage of motifs represents a total of twenty diverse context-dependent cholesterol interaction configurations. We envision that the gamut of cholesterol interaction sites, characterized by sequence plasticity in cholesterol interaction, could be relevant in receptor-cholesterol interaction in membranes of varying cholesterol content and organization, as found in diverse cell types. We conclude that an evolutionarily conserved mechanism of GPCR-cholesterol interaction allows the serotonin$_{1A}$ receptor to adapt to diverse membrane cholesterol levels during natural evolution.

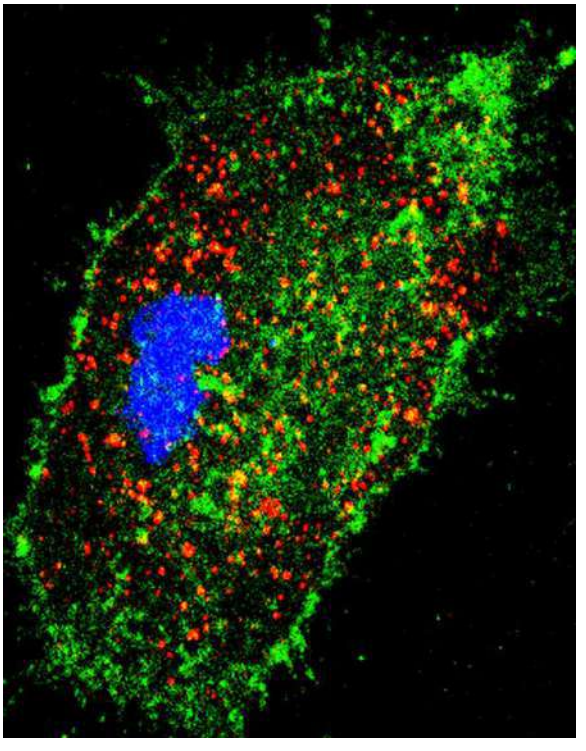


Fig. 1: Confocal microscopic image showing the serotonin$_{1A}$ receptor (green) undergoing agonist-induced endocytosis. The receptor co-localizes with transferrin (red), a marker for clathrin-mediated endocytosis.

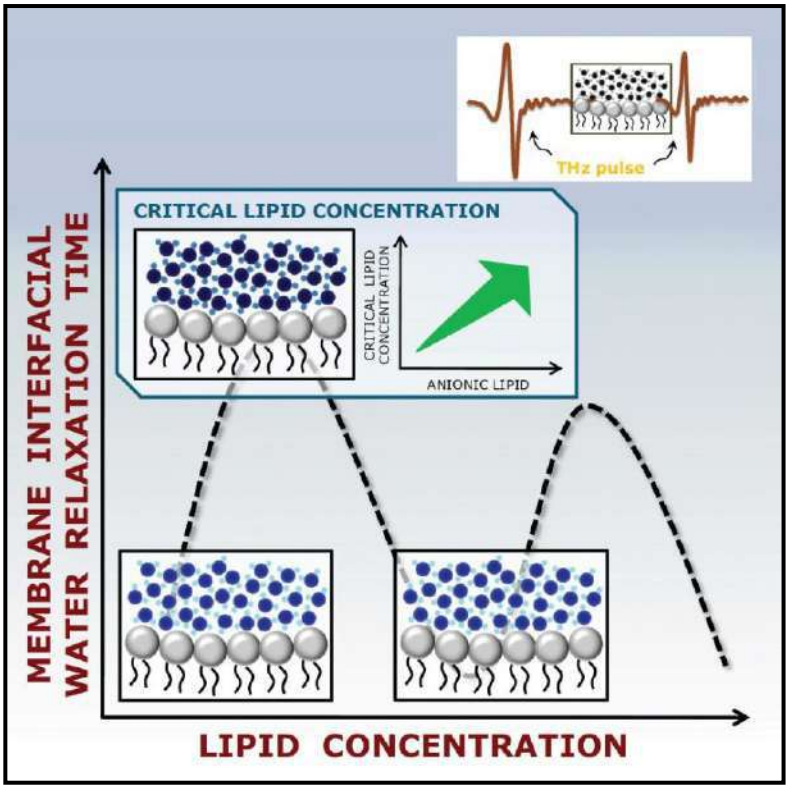


Fig. 2: A schematic representation of lipid concentration dependent "rise and dip" trend in hydration dynamics at the membrane interface, explored using terahertz time-resolved spectroscopy. The critical lipid concentration corresponding to this inflection point depends on phospholipid headgroup charge.

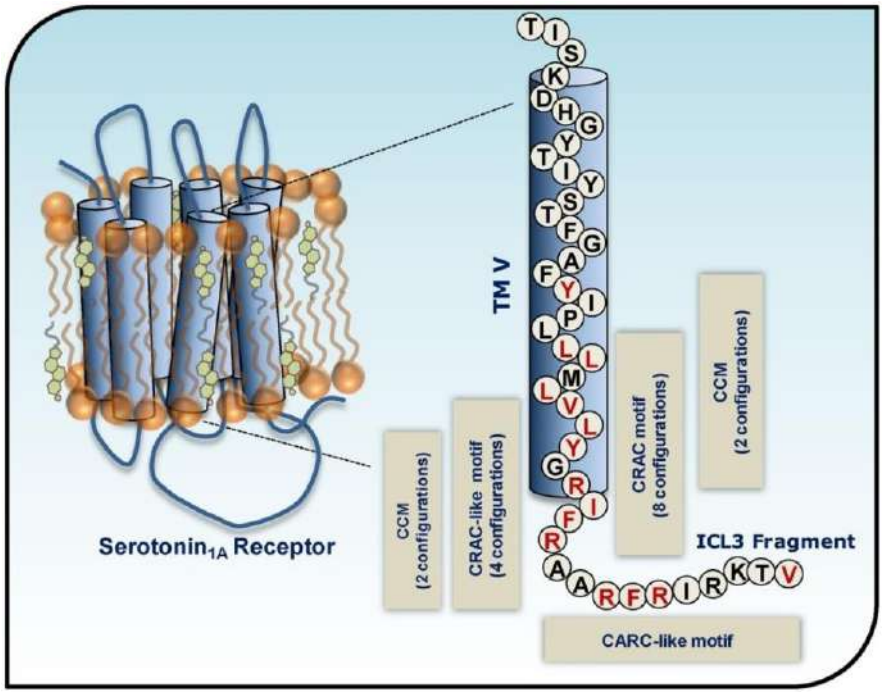


Fig. 3: Transmembrane helix V and intracellular loop 3 fragment of the serotonin$_{1A}$ receptor showing a conserved collage of putative cholesterol interaction motifs that enables the receptor to interact with membrane cholesterol in a variety of ways.

MANDAR V DESHMUKH

Molecular Basis of Evolutionary Divergence in RNAi Initiation

From left to right: K. Joy, Ramdas Aute, Sneha Paturi, Mandar V. Deshmukh, Jaydeep Paul, Upasana Rai, Sayali Khisty

RESEARCH INTERESTS:

- Structural biology of RNA binding proteins
- NMR methods and applications

"How do RNA-binding proteins impact post-transcriptional gene regulation? Our group is interested in understanding the role of regulatory proteins, which bind to a variety of RNA molecules and effect post-transcriptional gene regulation. We utilize solution NMR spectroscopy as a major tool together with complementary techniques in molecular biology, biochemistry, and biophysics."

Selected recent publications

- Rai U, Sharma R, Deshmukh MV (2018). Accessing structure, dynamics and function of biological macromolecules by NMR through advances in isotope labelling. *Journal of Indian Institute of Science* 16: 223-243.
- Chiliveri CS, Aute R, Rai U, Deshmukh MV (2017). DRB4 dsRBD1 drives dsRNA recognition in Arabidopsis thaliana tasi/siRNA pathway. *Nucleic Acids Research* 45: 8551-8563.
- Chiliveri CS, Deshmukh MV (2016). Recent Excitements in protein NMR: Large proteins and biologically relevant dynamics. *Journal of Biosciences* 41: 787–803.
- Sharma R, Sahu B, Ray MK, Deshmukh MV (2015). Backbone and stereospecific 13C methyl Ile (δ1), Leu and Val sidechain chemical shift assignments of Crc. *Biomolecular NMR Assignments* 9: 75-79.
- Chiliveri CS, Deshmukh MV (2014). Structure of RDE-4 dsRBDs and mutational studies provide insights in the dsRNA recognition in *C. elegans* RNAi. *Biochemical Journal* 458: 119-130.

We are interested in studying the diversity in the mechanism of non-coding RNA mediated gene regulation processes in higher eukaryotes. Non-coding regulatory RNA has emerged as a set of key molecules that are involved in gene regulation in a variety of cellular processes including development, infection, and cancer. In principle, the non-coding regulatory RNA modulates levels of cognate mRNA and thereby attenuating the expression of the corresponding gene. While the outcome of the non-coding RNA mediated gene silencing is nearly identical, the functional mechanism of non-coding RNA pathway has differently evolved in each organism. In prokaryotes, the non-coding RNAs shield the Shine-Dalgarno sequence responsible for the binding of the ribosome, whereas, in eukaryotes, they interact with the 3' untranslated region of mRNA which leads to translation inhibition or transcript cleavage.

Although we study non-coding RNA mediated gene silencing primarily in eukaryotes, our lab has previously determined the solution structure of Crc (*P. syringae* Lz4W) to understand its RNA binding property to elucidate its role in binding to specific sets of regulatory RNA, CrcY and CrcZ. Our studies revealed that Crc has divergently evolved from APendonucleases by losing its DNA binding activity and gained a completely new RNA interaction face. It further implied that the novel RNA binding face evolved in Crc is dynamic in nature and is tuned to bind both regulatory RNA as well as cognate mRNA so that it can regulate gene expression.

The landscape of non-coding RNA mediated gene regulation is much more diverse in higher eukaryotes where gene-specific precursor dsRNA elicits a response that results in post-transcriptional gene regulation, a process termed as RNA interference (RNAi). Depending on the nature and origin of trigger dsRNA the pathway is further classified as miRNA, siRNA or piRNA. RNA interference is induced as the immune response to foreign genetic material such as viral RNA as well as, in plants, to prevent the self-propagation of transposons. The RNAi pathway is initiated by the recognition of the precursor dsRNA by Dicer and its partner dsRNA binding protein, the dsRBP.

As mentioned in the previous annual reports, the Watson-Crick base pairing in a palindromic dsRNA – the precursor which is process by Dicer, predominantly assumes form-A of doublehelical nucleic acid structure. The arrangement imposes restrictions in the groove structures making minor groove shallower and major groove deeper and narrower, a feature that is recognized by a highly conserved dsRNA binding domains (dsRBD). A canonical dsRBD is comprised of the α-β-β-β-α structure where two α helices organize to form anα1α2 interface that packs against antiparallel β sheet formed by β1β2β3. dsRBDs recognize dsRNA through the helical face with three steric contacts in which residues Q/H and E in α1 contact minor groove, KKxxK belonging to the N-terminus of α2 bind to the major groove, and the H of the β1β2 loop contacts subsequent minor grove of dsRNA. The ability to identify exclusive structural features on dsRNA in an RNA sequence independent manner makes dsRBDs as the domain of choice by several important regulatory enzymes. Interestingly, Dicer's activity in processing precursor dsRNA in small interfering RNA is driven by its auxiliary proteins, dsRBPs (dsRNA Binding Domain Containing Proteins). For example, in *Drosophila*, Loquacious associates with Dcr-1 for miRNA-mediated silencing, and R2D2 forms a stable complex with Dcr-2 to initiate siRNA-mediated silencing. Four Dicer-like proteins of *A. thaliana* interact with five dsRBPs (HYL1, DRB2 to DRB5). Human Dicer requires dsRBPs such as TRBP and PACT for miRNA mediated gene silencing. In *C. elegans*, the dsRBP, RDE-4 promotes Dicer-1 during the initiation of RNAi. One of the key features of dsRBPs is the presence of two or three dsRNA binding domains that bear significant sequence homology across various organisms and are connected by a linker heterogeneous in length. Despite similarities, dsRBPs are seen to exhibit a wide spectrum of affinities with dsRNA. In a few cases, loss of dsRNA affinity endows them to involve in other functions such as protein:protein interaction. Several dsRBPs either co-elute with the

corresponding Dicer or constitute a homodimer for efficient recognition of the substrate. In the case of TRBP, the C-terminal region folds into a canonical dsRBD and is essential to bind the Helicase domain of Dicer to activate miRNA response. However, other homologs do not possess any sequence similarity with TRBP in the C-terminal region, which implies that other dsRBPs interact with respective Dicers differently. Importantly, several prominent changes in the key features in dsRBPs, such as modification to the dsRBD fold, loss of functionality of dsRBD, differences in the C-terminal regions, and dimerization of dsRBPs can be further noticed.

In the last ten years, we have solved the solution structure of RDE-4, which initiates RNAi in *C. elegans*, as well as DRB4 which regulates efficient tasi/si RNA dependent viral defense response in *A. thaliana*. For RDE-4, we showed that both dsRBDs possess additional structural elements that are responsible for the dsRNA binding ability and part of the linker and dsRBD2 alone are necessary and sufficient for RDE-4's ability to initiate RNAi by the association with Dcr-1 and dsRNA. In the case of DRB4, we showed that the key structural and dynamic features in dsRBD1, as well as the unique nature of the linker, are important for the successful outcome of the tasi/siRNA pathway in plants.

The microRNA pathway in *Arabidopsis* is involved in regulating many more genes than other eukaryotes, as mutations in RNAi genes caused phenotypic characters. Moreover, the majority of genes regulated by miRNAs in plants are transcription factors. The miRNA pathway is carried out by DCL1:DRB1 which is primarily involved in miRNA mediated mRNA cleavage and under stress, DRB2 supersedes DRB1 to bind with DCL1 and affects miRNA driven translation inhibition. DRB2 has been also shown to negatively regulate levels of DRB1 mRNA so that the miRNA driven translation inhibition pathway is selected unhindered. The ability of plants to choose between cleavage and translation inhibition is remarkable and is in quite a contrast with animals where differences and mismatches in pre-miRNA regulate between the cleavage or translation inhibition of cognate mRNA.DRB2 is a multimeric protein of ~47 kDa (434 amino acids) and based on primary sequence comparison with previously studied dsRBDs three regions were predicted for DRB2, which include two N-terminal dsRNA binding domains (1-70 and 87-155) separated by an 18 residue long linker (71-86) and a long C-terminal region (156-434) with no structural homology. DRB2 WT has interspersed six PxxP motifs that perhaps mediate protein:protein or protein-membrane interactions. Based on residue comparison and secondary structure prediction three truncated constructs of DRB2 were designed. During the previous year, we have solved the solution structure of the first dsRBD of DRB2 and showed that it assumes a canonical α-β-β-β-αstructure. The structure also indicated a well-conserved dsRNA binding patch that is identical to its homologs.

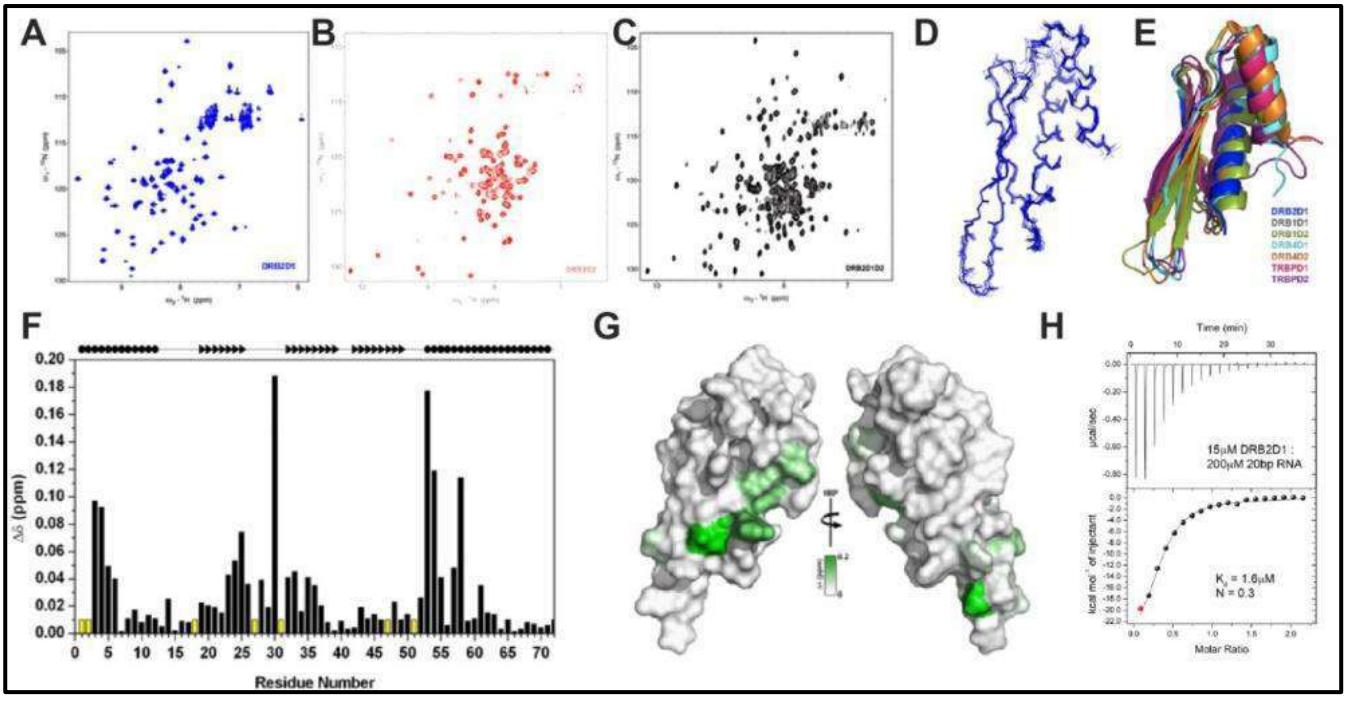


Fig. 1: Structural studies on DRB2 to understand miRNA biogenesis in plants. (A) ^{15}N-^{1}H HSQC of DRB2D1, (B) ^{15}N-^{1}H HSQC of DRB2D2, (C) ^{15}N-^{1}H HSQC of DRB2D1D2, (D) Ensemble structure of DRB2D1, (E) Overlay of lowest energy structure of DRB2D1 with its homologues, (F) Chemical shift perturbation in DRB2D1 upon titration with^{15}N DRB2D1, (G) Map of chemical shift perturbations on the structure of DRB2D1, and (H) ITC isotherm of DRB2D1 binding with 20 bp dsRNA.

During this year, we have explored structural studies on dsRBD2 and dsRNA binding studies on both domains of DRB2. The multiple constructs of DRB2 dsRBD2 made previously did not yield a stable domain, hence, we made an additional construct with a C-terminal extension for the dsRBD2. Figure 1 shows the ^{1}H-^{15}N HSQC of DRB2D2, DRB2D2, and DRB2D1D2 which shows their folded nature. Interestingly the spectral broadening seen for dsRBD2 in the case of DRB2D2 and DRB2D1D2 (Fig. 1B and 1C) indicates that the dsRBD2 is either partially folded state (the molten globule state) or oligomeric state. The ^{15}N backbone relaxation studies, analytical ultra-centrifugation, and small angle X-ray scattering data further indicated that the dsRBD2 is indeed in a partially folded state. Further, the solution structure of DRB2D2 was determined using conventional NMR methods such as NOESY, RDCs, and torsion angles and refined to the ensemble R.M.S.D. of ~ 0.6 Å (Fig. 1D). The structure suggests that DRB2D1 assumes a canonical dsRBD fold and also displays a very high degree of structural homology with several dsRBDs with backbone R.M.S.D. of ~ 1.5 Å (Fig. 1E). To map the dsRNA binding region in DRB2D1, we have performed NMR and ITC based titration of 13 bp and 20 bp dsRNA, respectively. The chemical shift perturbation observed by NMR (Fig. 1 F) was mapped in the lowest energy structure of DRB2D1 (Fig. 1G) which indicates an intermediate timescale dsRNA:dsRBD interaction. The ITC binding isotherm also indicate that DRB2D1 interacts with 20 bp dsRNA binding with a relatively weaker affinity. To explore, RNA binding activity of both domains with longer dsRNA and functionally relevant substrates (such as miR169), we have performed EMSA assays (Fig 2). The data indicate that the DRB2D1 lacks binding activity with structured as well as longer dsRNA (Fig. 2A and 2D), whereas DRB2D2 binds to both dsRNA with relatively higher affinity (Fig. 2B and 2E). Interestingly, DRB2D1D2 that contains both dsRBDs and the linker binds to both dsRNA (Fig 2C and 2F) with features similar to DRB2D2 suggesting that the DRB2D2 is the principle dsRNA recognizer in DRB2.

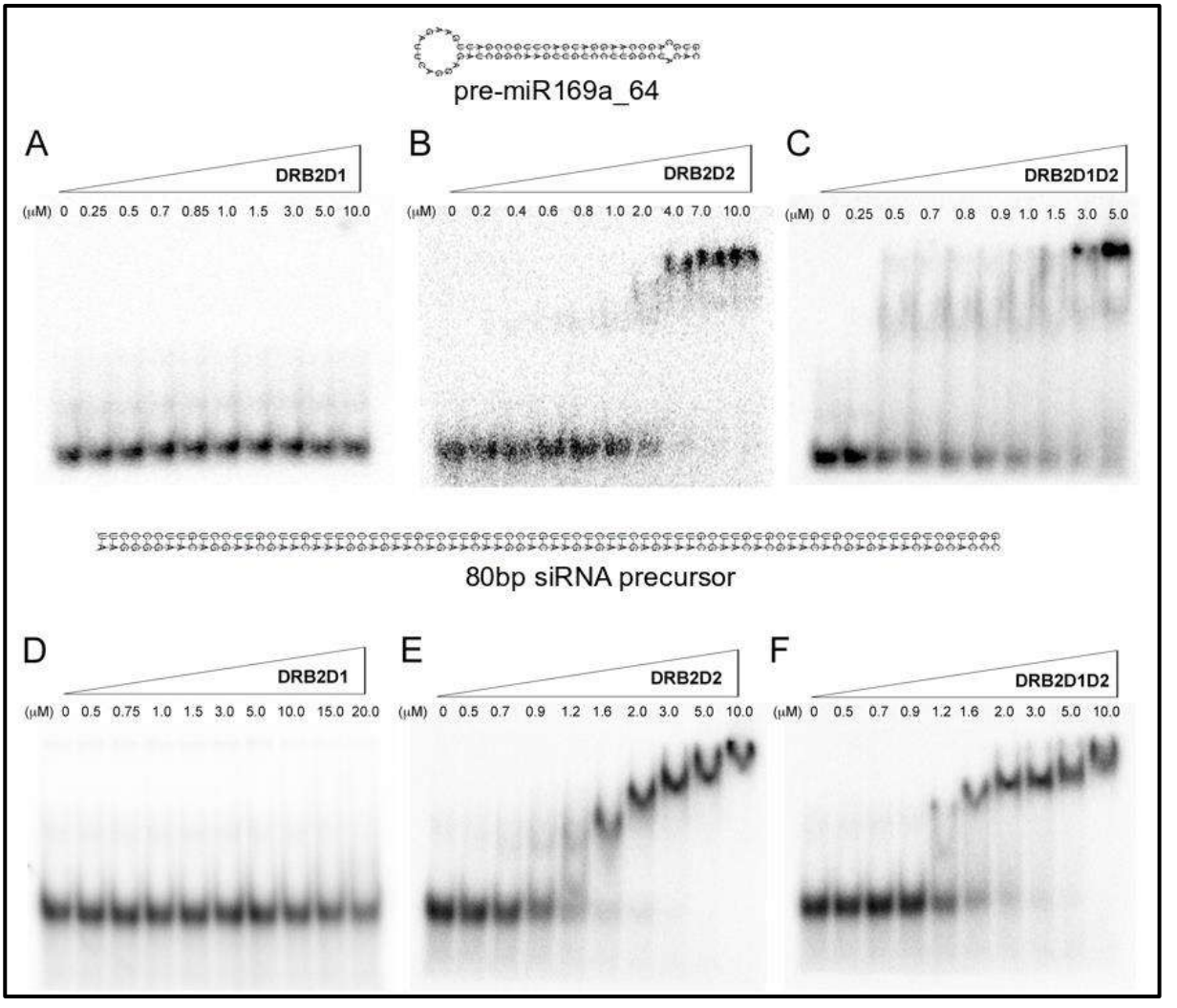


Fig. 2: EMSA assays for titration of dsRNA binding of various constructs of DRB2 with miR169 and 80bp dsRNA. (A) DRB2D1 with miR169, (B) DRB2D2 with miR169, (C) DRB2D1D2 with miR169, (D) DRB2D1 with 80 bp dsRNA, (E) DRB2D2 with 80 bp dsRNA, (F) DRB2D1D2 with 80 bp dsRNA.

JYOTSNA DHAWAN

Molecular programs of quiescence in adult stem cells and regeneration

From left to right: (sitting): Lamuk Zhaveri, Ranjitha Boopathy, Ananga Ghosh, Gunjan Purohit, Manjit Rana, Devesh Bahety, Debarya Saha (standing) Prabhavathy Devan, Nandini Rangarajan, Ajoy Aloysius, A.S. Priti, Jyotsna Dhawan, Abiroop Dey, Sujoy Deb, Kapila Awasti, Swetha Sundar

RESEARCH INTERESTS:

- Control of cellular quiescence and its relationship to stem cell function
- Adult stem cells and skeletal muscle regeneration
- Epigenetic, transcriptional and post-transcriptional mechanisms in quiescence
- Secreted and mechanical signals in control of cell fate

"We are interested in the mechanisms by which the dormant or quiescent state of adult stem cells promotes the acquisition and maintenance of regenerative function. We use genome-wide strategies coupled with functional analysis in muscle cell lines and primary stem cells to investigate the links between two key features of quiescence - protection from differentiation and the potential to return to active division."

Selected recent publications

- Saleh A, Gunasekaran S, Raychaudhuri S and J Dhawan (2019). Cytoplasmic sequestration of the RhoA effector mDiaphanous1 by Prohibitin2 promotes muscle differentiation. *Scientific Reports* 9: 8302. .
- Aloysius A, Das Gupta R, Dhawan J (2018). Lef1 switches partners from β-catenin to Smad3 for transcriptional activation of Tcf/Lef1 target genes in quiescent muscle stem cells. *Science Signaling* 11: 540.
- Zaveri L, Dhawan J (2018). Cycling to Meet Fate: Connecting pluripotency to the cell cycle. *Frontiers in Cell Development Biology* 6: 57.

Preprints:

- Gala HP, Saha D, Venugopal N, Aloysius A, J Dhawan (2018). RNA polymerase II pausing regulates a quiescence-dependent transcriptional program, priming cells for cell cycle reentry. *bioRxiv* 250910.
- Harvey RP, Asli N, Xaymardan M, Forte E, Waardenberg AJ, Cornwell J, Janbandhu V, Kesteven S, Chandrakanthan V, Malinowska H, Reinhard H, Yang S, Pickett H, Schofield P, Christ D, Ahmed I, Chong J, Heffernan C, Li J, Simonian M, Bouveret R, Srivastava S, Mishra RK, J Dhawan, Nordon R, Macdonald P, Graham

R, Feneley M (2018). PDGFRα signaling in cardiac stem and stromal cells modulates quiescence, metabolism and self-renewal, and promotes anatomical and functional repair. *bioRxiv* 225979.

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

Using myogenic cell lines, muscle stem cells (Figure 1) and mesenchymal stem cells we have described active controls at multiple levels of gene regulation specific to quiescence. Our studies indicate that quiescent cells preserve two antagonistic programs (division vs. differentiation) in an inactive but poised state that is rapidly reversed during cell cycle reentry.

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

Gene regulatory mechanisms in G_0

Earlier we reported that promoter-proximal pausing of RNA pol II is involved in the maintenance and exit from the quiescent self-renewing state (Gala *et al*, bioRxiv, 2018). Over the past year we investigated regulators of Pol II function in the transcription cycle using siRNA knockdown, and found that well known regulators such as NELFb, Hexim and Brd4 do not appear to affect stalling or the kinetics of cell cycle re-entry from G_0. Unexpectedly, the positive elongation factor Aff4 appears to play a non-canonical role in quiescence: compromising its expression leads to more rapid cell cycle re-entry, suggesting that this regulator places constraints on elongation during G0. Our ongoing studies use knockdown as well as peptide-mimetic inhibitors of Aff4 (synthesized by Dr Praveen Vemula, inStem), to dissect the altered mechanism by which Pol II pausing is controlled at the G_0-G_1 transition.

Rho signaling and muscle differentiation

Cytoskeletal signaling is integrated with growth factor signaling via the activity of the RhoAGTPase. Earlier we had identified mDiaphanous (mDia) as the key effector of RhoA in mediating signaling via regulation of transcription factors that are responsive to the polymerization state of the actin cytoskeleton (Gopinath*et al*, J. Cell Sci, 2007). We now report that mDia negatively regulates myogenesis at the level of Myogenin expression. In myotubes, over-expression of mDia1ΔN3, a RhoA-independent mutant, suppressed Myogenin promoter activity and expression. Using yeast two-hybrid and mass-spectrometric analysis we investigated mDia1-interacting proteins that may counteract its suppressive function to permit Myogenin expression and timely differentiation. We found that mDia1 has a stage-specific interactome, including Prohibitin2 (Phb2), MyoD, Akt2, and β-Catenin, along with a number of proteosomal and mitochondrial components. Of these interacting partners, Phb2 co-localises with mDia1 in cytoplasmic punctaeonly in myotubes. Using domain mapping, we dissected the functional consequences of this partnership on Myogenin promoter activity, andfind that Phb2 sequesters mDia1, dampens its anti-myogenic activity and fine-tunes RhoA-mDia1 signalling to promote differentiation. Thus mDia1 is multi-functional signalling effector whose anti-myogenic activity is modulated by a differentiation-dependent interactome (Saleh et al, *Sci. Rep*., 2019).

Developing metabolite profiles of different muscle cell states

It is now well documented that the rise in incidence of metabolic syndrome is associated with excessive consumption of saturated fat. Diet affects a variety of tissue functions and adaptations. Our objective is to understand the effect of diet-dependent metabolic transitions on muscle stem cells and regeneration. Earlier we had reported our preliminary results showing that mice were fed with high-fat diet for 2 months exhibit diminished muscle stem cell function and poor regeneration. To understand the mechanism by which altered metabolic states affect stem cells, we are currently establishing the metabolic profiles of cultured muscle cells in proliferating, quiescence, reactivated and differentiated conditions. We have used two methods: first, in collaboration with Dr Anant Patel at CCMB, we have evaluated metabolite profiles using NMR. Second, in collaboration with Dr. Siddesh Kamat at IISER Pune, we are using LC-MS/MS to profile a wider range of metabolites. Both methods have yielded patterns typical of individual cellular states, and studies are ongoing to evaluate causal relationships of specific metabolite groups with distinct cellular states.

Developing enabling technologies for *in vitro* cultured meat

Livestock farming and the meat industry are increasingly viewed as unsustainable due to the negative impact of this activity on the environment and climate change, given that the number of agricultural animals far exceeds the number of humans on our planet. Cultured meat has been proposed as a futuristic alternative to traditional meat production, and several efforts worldwide are attempting to establish methods and scale up for expansion of muscle cells as the basis for in vitro meat production. Towards that end, and based on the experience of CCMB in muscle cell culture, the lab has been tasked by the DBT with exploring the possibility of culturing muscle cells (myoblasts) from livestock species. This project has been initiated in collaboration with Dr. G Patil at the ICAR-National Center for Research on Meat in Hyderabad, and includes three groups at CCMB (N. Madhusudhana Rao, J.Dhawan and V. Radha). The objectives are to establish methods for isolation, culture and expansion of myoblasts from sheep muscle and evaluation of the feasibility of expansion at a scale that would be needed for commercial production. We have initiated activities in culturing and establishing the basic parameters for efficient expansion of primary sheep myoblasts, including their growth and differentiation properties (Figure 2).

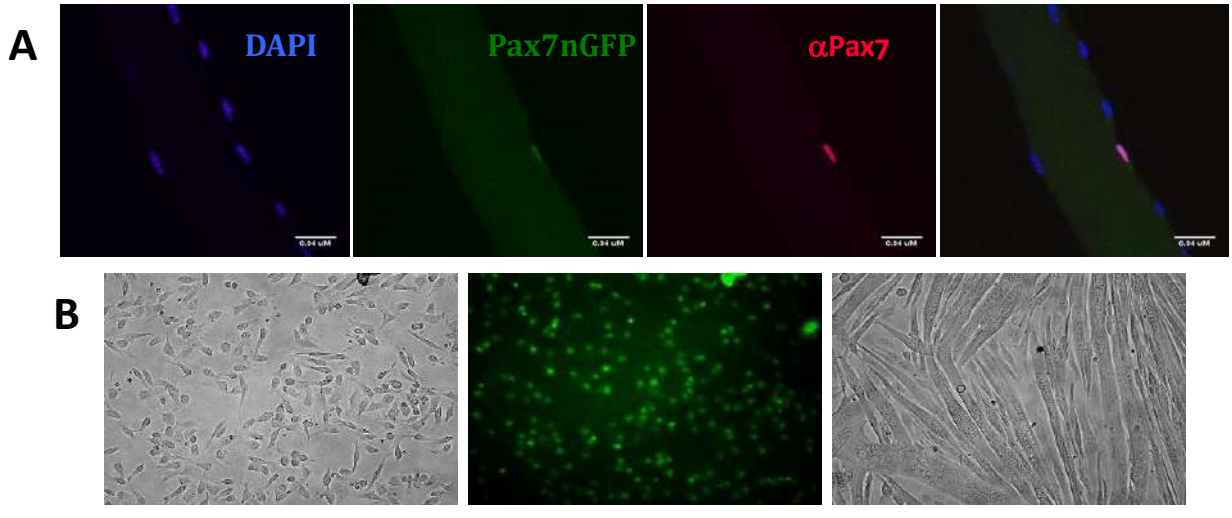


Fig. 1: Muscle stem cells from mice. (A) A single myofiber isolated from Tg:Pax7ngfp mice and placed in culture: note the GFP+ (green) muscle stem cells in their niche, stained with an anti-Pax7 antibody (magenta) (b) Activated muscle stem cells proliferating in culture (left panel) continue to express Pax7 (middle panel). Right panel shows muscle stem cells triggered to differentiate and fuse to form multinucleated myotubes that recapitulate the molecular and regulatory features of myofibers *in vivo*.

Fig. 2: Differentiated skeletal muscle myotubes derived from primary sheep muscle stem cells (day 5 of differentiation). Note the long fused syncytial (multinucleated) myotubes.

G UMAPATHY

Understanding Species Extinction and Conservation Physiology

From left to right: Deepanwita Purohit, S.Manu, Sanjay Balakrishnan, Mihir Trivedi, Caroline Karan, G.Umapathy, K. Hariharan, Vinod Kumar, Nandini Muthivelan

RESEARCH INTERESTS:

- Understanding species extinction process in human dominated landscape
- Conservation breeding and conservation physiology
- Genomics in biodiversity monitoring and conservation

"Our group aims to understand factors that influence the extinction of species in the human-dominated landscape, to monitor and assess aquatic biodiversity using eDNA and to assess genetic and reproductive fitness in wild animals"

Selected recent publications

- Tyagi A, Kumar V, Kittur S, Reddy M, Naidenko S, Ganswindt A, Umapathy G (2019). Physiological stress responses of tigers due to anthropogenic disturbance especially tourism in two central Indian tiger reserves. *Conservation Physiology* 7: coz045.
- Purohit D, Ram MS, Pandey V, Pravalika S, Deka PJ, Narayan G, Umapathy G (2019). Cross-specific markers reveal retention of genetic diversity in captive-bred pygmy hog, a critically endangered suid. *Conservation Genetics Resources* https://doi.org/10.1007/s12686-019-01091-1.
- Budithia NMR, Kumar V, Yalla SKa, Rai U, Umapathy, G (2016). Non-invasive monitoring of reproductive and stress hormones in the endangered red panda (*Ailurus fulgens fulgens*). *Animal Reproduction Science* 172: 173–181.
- Chakraborty D, Hussain S, Reddy DM, Raut S, Tiwari S, Kumar V and Umapathy G (2015). Mammalian gastrointestinal parasites in rainforest remnants of Anamalai Hills, Western Ghats, India. *Journal of Biosciences* 40 399–406.
- Kumar, V, Reddy, V, KokkiligaddaA, Sivaji, S, Umapathy, G. (2014).Non-invasive assessment of reproductive status and stress in captive Asian elephants in three south Indian zoos. *General and Comparative Endocrinology* 201: 37-44.

Patents Filed

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Physiological stress responses of tigers due to anthropogenic disturbance especially tourism in two central Indian tiger reserves

The tiger (*Panthera tigris*), is an endangered species that has lost more than 95% of its global historical home range, and its extant population now exists in fragmented habitats across its former area. Despite steep declines in population size and habitat, the Indian subcontinent remains a key area for tiger conservation as it harbours around 60% of the current global free-roaming tiger population. However, tigers continue to suffer from several anthropogenic threats like poaching, habitat loss and fragmentation. It has been shown that human disturbance, as well as tourism pressures, can act as potential stressors for wildlife; evoking physiological stress and fitness responses.

Our study examines the physiological stress response of tigers due to anthropogenic activities including wildlife tourism in Bandhavgarh (BTR) and Kanha Tiger Reserve (KTR) using fecal glucocorticoid metabolite measurement. We collected a total of 341 faecal samples from both reserves during tourism and non-tourism periods. Data on various anthropogenic disturbances including tourism activities, like number of vehicles and visitors (Fig. 1) was also collected. We ascertained the species identity and sex of all the samples collected using genetic markers. Faecal glucocorticoid metabolites (fGCM) were extracted using a previously reported procedure andfGCM concentrations subsequently determined using an established enzyme immunoassay.

In total 2,44,179 people visited both BTR (106,535) and KTR (1,37,644) during the nine months of tourism season (October 2014 to June 2015), with an average of 395 people/day in BTR, and 509 people/day in KTR. To travel inside the parks, 51,695 vehicles have been used in total (BTR: 23011; KTR: 28684; Fig 1) during the study with an average of 85 and 106 vehicles/day in BTR and KTR, respectively. We collected 341 suspected tiger faecal samples in total, of which 206 samples (BTR: 114; KTR: 92) were identified to be samples from tigers.

Fig. 1: Tourist vehicles go after the tiger and several times excessively close to it

Overall mean fGCM concentrations of tigers roaming at BTR (51.45 ± 4.75 ng/g) and KTR (56.46 ± 6.6 ng/g) were not significantly different (M-W Test, n=206, P=0.87). We found significantly higher fGCM concentrations in tigers at BTR during the tourism period (56.47 ± 5.81 ng/g, n=91) compared to the non-tourism period (32.69 ± 2.76 ng/g n=23; M-W Test, n=114, P=0.001 Fig 2a). Similarly, fGCM concentrations showed a positive correlation with the number of vehicles visited per day during the tourism period (r=0.34; P=0.001; n=91). There were no significant differences in fGCM concentrations between the sexes during tourism and non-tourism period (M-W Test, n=91, P=0.15 and n=23, P=0.56). GLM results showed that fGCM concentrations are significantly influenced by tourism season (F_1=4.710; P=0.032), number of vehicles (F_4=3.97; P=0.010), and disturbance level (F_3=6.62; P= 0.0001). Sex and sample location (core and buffer) did not influence fGCM concentrations determined during the study period (GLM $F_{1=}$ 0.13; P=0.60 and $F_{1=}$ 0.033; P=0.75, respectively). Similarly, we found significantly higher fGCM concentrations in tigers at KTR during the tourism period (64.09 ± 7.88 ng/g; n=92) compared to the non-tourism period (22.82 ± 2.54 ng/g n=17; M-W Test, n=109, P=0.001; Fig 2b). GLM analysis showed that fGCM concentrations are significantly influenced by tourism (F_1= 10.07; P=0.001) (Fig. 2)

Understanding the impact of anthropogenic stressors on tiger populations can provide valuable information for optimizing conservation and management strategies. Our study showed that wildlife tourism can cause distinct physiological stress in tigers in protected areas. We demonstrate that tourism and thus anthropogenic disturbance are correlated with fGCM concentrations of tigers in both monitored reserves. Although both reserves experience a similar tourism pressure, the stronger correlation found in BTR might be attributed to the comparatively higher number of human settlements and cattle densities in and around the reserve. Since, the tigers at BTR are genetically less connected to other populations as the ones at KTR, conservation efforts should even focus on the BTR population.

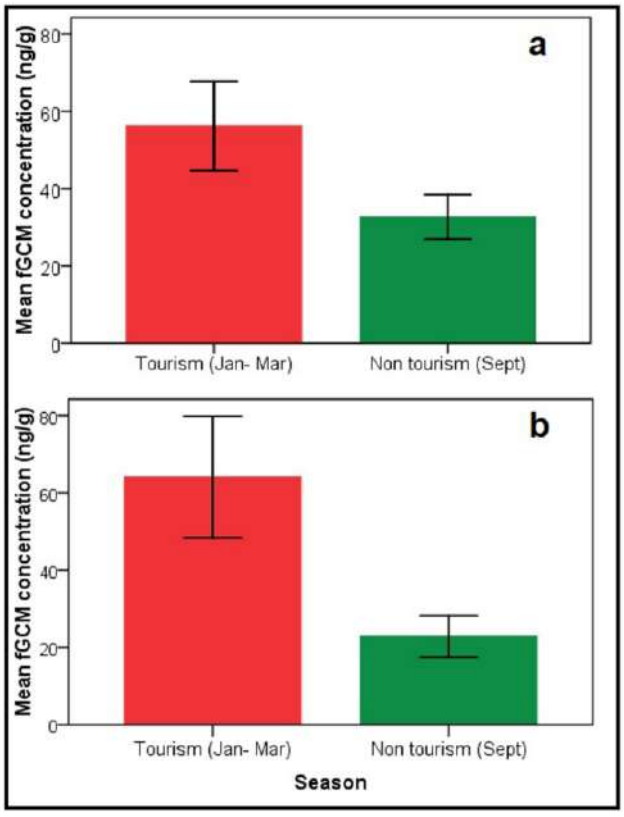


Fig. 2a & b: Mean (± SEM) fGCM concentrations in tigers during the tourism (Jan-Mar) and non-tourism (Sept) seasons in BTR and KTR

However, as our study only provides a snapshot of the effects of anthropogenic disturbance on tiger, long-term individual based studies with greater spatial and temporal sampling would be crucial to better understand the adverse effects of anthropogenic stressors on the physiology of this keystone species. Our management recommendations include strict regulation of vehicular traffic and tourists per vehicle, shifting of artificial waterholes away from tourist roads, and reducing other anthropogenic disturbance, including relocation of villages from the core area of tiger reserves.

Development genetic marker for species / sex identification and assessment of genetic diversity in captive-bred pygmy hog - a critically endangered suid

The critically endangered wild suid, pygmy hog (*Porculasalvania*), represents the rarest and smallest member of the Suidae family. It once occupied the tall

and wet grasslands throughout the southern Himalayan foothills in India but became locally extinct from most parts of its original range due to habitat loss and degradation. To avert further extirpation, a conservation-breeding project, the Pygmy Hog Conservation Program (PHCP), was started in 1995 with six founding individuals captured from one of the last surviving populations, with an intent to breed and repopulate pygmy hog in sites from which it had disappeared. The program has been immensely successful in the captive breeding and release of 110 individuals into the wild (Fig. 3).

Fig. 3: Pygmy hogs at conservation breeding centre Guwahati, Assam. Photo credit Tammo Buss.

Although the PHCP has benefited from several years of planned breeding and pedigree management, a lack of genetic information about the breeding individuals may lead to unforeseen decline in genetic pool and associated detrimental effects in captive and reintroduced populations. Hence, periodic genetic assessment of the captive population is of paramount importance to the long-term success of PHCP. Furthermore, non-invasive genetic monitoring is an indispensable tool for the evaluation of reintroduction success in pygmy hog. The objectives of the present study were to assess the genetic diversity of a captive bred, population of pygmy hog using a set of polymorphic microsatellite markers, and to develop molecular markers for non-invasive, species and sex identification.

Fresh faecal samples (n = 6) and cartilage tissue from ear punches (n = 19; 4 of them from founder population) of live pygmy hogs, and muscle tissue (n = 9) of dead pygmy hogs were collected from the captive population at PHCP conservation-breeding and research centre in Basistha, Assam. We tested thirteen microsatellite markers for polymorphism in pygmy hog using 34 faecal and tissue samples. The result showed that ten markers were polymorphic with 3–9 alleles per locus (mean = 5.8). Using these markers, we generated the genotypes for 30 individuals. The observed individual heterozygosities ranged from 0.32 to 0.85. After six generations of planned breeding from six founder individuals, the captive pygmy hog population at PHCP, Basistha showed an HE of 0.603. To check if the genetic diversity is maintained across generations of captive-bred population, we assigned individuals into seven generations including the founder generation (Gen0). Gen2 and Gen3 were excluded from the statistical analysis as they were only represented by a single sample. We found that the allele diversity was stable between generations.

The ten polymorphic loci reported in the present study could also be used for the post-release monitoring of pygmy hog individuals using non-invasive samples. The cumulative probability of identity (PID) and the probability of identity for siblings (PID(sibs)) for the marker set were found to be $1x10^{-8}$ and $7.2x10^{-4}$respectively. Amplification using a novel species-identification marker PsF1/PsR1 produced an amplicon of length 320 bp. Sequencing of PCR product confirmed its identity as CytB. Furthermore, PCR using faecal DNA of *Sus scrofa*and other sympatric ungulates resulted in non-specific bands, validating the pygmy hog-specificity of the marker. Meanwhile, the novel sex-determining marker SdSRY amplified a single product of size 202 bp in male pygmy hogs, whereas no amplification was seen in females.The molecular markers developed and tested in the current study would be extremely useful for the post-release monitoring of pygmy hogs during reintroductions.

AJAY GAUR

Conservation Genetics of Endangered Species

From left to right: Paridhi Singh, Yamuna Perumalsamy, Ajay Gaur, Ara Sreenivasu, Mohini Bhandare, Ritu Sharma

RESEARCH INTERESTS:

- Population Genetics
- Evolutionary Genetics
- Wildlife Forensics
- Conservation Breeding
- DNA Banking

"Our group focuses on the development and application of molecular markers in conservation genetics of Indian endangered species"

Selected recent publications

- Mitra S, Sreenivas A, Sowpati DT, Kumar AS, Awasthi G, Kumar M, Tabasum W, Gaur A (2019). De novo assembly and annotation of Asiatic lion (*Panthera leo persica*) genome. *BioRxiv*: doi: https://doi.org/10.1101/549790.
- Gaur A, Parmar DR (2018). How healthy the Indian leopards are? *Atlas of Science*: 25th July, http//atlasof science.org.
- Sarvani RK, Parmar DR, Tabasum W, Thota N, Sreenivas A, Gaur A (2018). Characterization of complete mitogenome of Indian mouse deer, *Moschiolaindica* (Artiodactyla: Tragulidae) and its evolutionary significance. *Scientific Reports* DOI: 10.1038/s41598-018-20946-5.
- Kunteepuram V, Sreenivas A, Tabasum W, Challagandla AK, Gaur A (2018). The complete mitochondrial genome of Asian palm civet (*Paradoxurus hermaphrodites*) with phylogenetic consideration. *Mitochondrial DNA part B* DOI: 10.1080/23802359.
- Tabasum W, Parmar DR, Jayaraman A, Mitra S, Sreenivas A, Kunteepuram V, Gaur A (2017). The complete mitochondrial genome of Eld's deer (*Rucervuseldiieldii*) and its phylogenetic implications. *Gene Reports*: DOI: dx.doi.org/10.1016/j.genrep. 2017.10.001.

Wild animal populations that once were large and widespread have become small and fragmented due to habitat loss, geographical fragmentation and other anthropogenic interferences. Small populations face greater demographic and genetic risks. Conservation genetics deals with genetic management of small populations, resolution of taxonomic uncertainties and the use of molecular genetic analysis in forensics and understanding of species biology. We focus on the development and application of molecular markers in conservation genetics of endangered species. The major efforts are towards the use of non-invasive sampling protocols and development of species–specific DNA markers to look into the genetic structure of existing populations. We have developed several polymorphic microsatellite and mitochondrial markers in big cats, ungulates, primates and other endangered Indian species.

Whole genome de novo sequencing of Asiatic lion (*Panthera leo persica*)

The Asiatic lion, *Pantheraleopersica*, is one of the most endangered large mammals in the world. A small lion population in the Gir forest of India, estimated at 523 (Gujarat Forest Department Census 2015), represents the only known remaining wild population of an animal that historically ranged throughout most of the south eastern and south western Asia, extending from Syria to north India, as recently as 200 years ago. The subspecies became extinct in Syria, Iraq, Iran, Afghanistan and Pakistan in the later part of the 19th century. In India too, the lions were once spread across Rajasthan, Gujarat, Haryana, Punjab, Uttar Pradesh, Madhya Pradesh and western Bihar.Population pressure and extensive hunting for trophies reduced the Asiatic lions alarmingly less in number.With strengthening conservation measures and habitat recovery by incessant efforts of the Indian state of Gujarat, the number of lions has increased significantly in the Gir forest. However, with the entire wild population being confined to a single location, the Asiatic lion is facing an increased risk of extinction because of a very low number and continuous inbreeding. Morphological and molecular approaches have been used to study genetics, population structure and evolutionary history of this. The molecular genetic assessment ofthe Asiatic lions at whole genome level can be utilized for designing further conservation and management strategies for this critically endangered Indian big cat.

a. Genome sequencing and de novo assembly

The whole genome sequence of one male Asiatic lion (*Panthera leo persica*) from Nehru Zoological Park, Hyderabad was determined by constructing a total of three paired-end libraries followed by sequencing in an Illumina HiSeq 2500. Mate-paired libraries were also prepared and sequenced to increase coverage and to span repetitive sequence elements. After filtering the raw reads a draft genome assembly of Asiatic lion was then prepared. The assembled genome of Asiatic lion was mapped against the cat reference genome (*Feliscatus* – 8.0) and was found to have a similarity higher than 95% with 19 chromosomes and about 49.8 million homozygous and heterozygous variants (including SNVs and Indels). In order to ensure a high quality of assembly, the lion blood transcriptome was sequenced from five individuals. The lion assembly was aligned to the lion blood transcripts and cat ESTs revealing > 81% coverage and >86% mapping rate respectively.

In total, we generated 231Gb sequence data with the read lengths of 100 bp and 250 bp for pair-end and mate-pair libraries respectively, achieving a total of 90X fold coverage of the whole genome. To ensure an accurate assembly, we excluded data from poor libraries, eliminated low-quality reads with erroneous bases or other ambiguous sequence data, and used 152 Gb (62X) high quality reads for *de novo* assembly and an estimated genome size of 2.3 Gb by using frequency of 23-mer reads. The resulting assembly scaffolds have an N50 of 20,864, the longest scaffold being 383,982 Kb.

b. Annotation of Asiatic lion genome

The assembled Asiatic lion genome was annotated to delineate tandem repeats, predict genes and gene functions and detect non-coding RNAs. The pattern of repeat distribution in the genome was similar to that of the cat and Amur tiger with LINEs forming the largest proportion in all the three genomes (Figure 1).

Gene prediction analysis was then performed to delineate protein-coding genes and non-coding RNAs in the Asiatic lion.

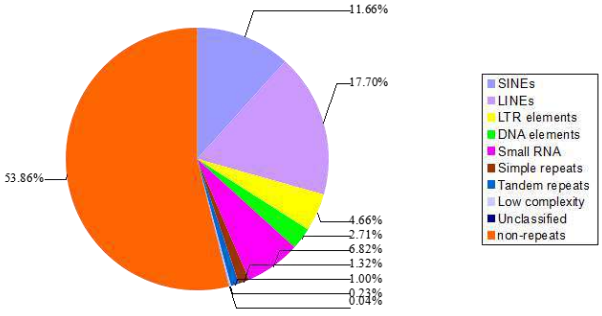


Fig. 1: Distribution of repeats in the Asiatic lion genome

The lion proteome was reconstructed by comparison of genomes of eight mammals (tiger, cat, dog, panda, opossum, human, mouse). A comparison between Asiatic lion, Amur tiger, cheetah and cat was also done to determine Felidae-specific and species-specific protein families. Finally, the protein families specifically expanded and contracted in Asiatic lion were determined.

c) Genetic diversity of Asiatic lion

Felids, in the order Carnivora, have distinctively small populations and are also typified by low genetic diversity which holds pertinence in their conservation. Even within felids, the Asiatic lion lies towards one end of the genomic diversity spectrum, as indicated in a graph showing the rate of heterozygous SNPs per base pair of a genome (Figure 2). These statistics have also been used as the index of genomic diversity in other whole genomes published. Mapping Asiatic lion raw reads to the cat genome unravelled 745,184 SNPs and a comparison with genome size gave a rate of 0.000276 heterozygous SNVs per base pair.Thisis lower relative to the other two sub-species of lion, African lion and white lion and comparable with the Eurasian lynx which is also facing the risk of extinction.The rate is alsomuch lower than the average (0.00094) reported for Felidae genomic diversity. The low genomic diversity characterized by low rate of heterozygous SNPs per base pair can be attributed to the low effective population size of Asiatic lion in wild but a genetic investigation with multiple Asiatic lion individuals will be necessary to confirm this finding.

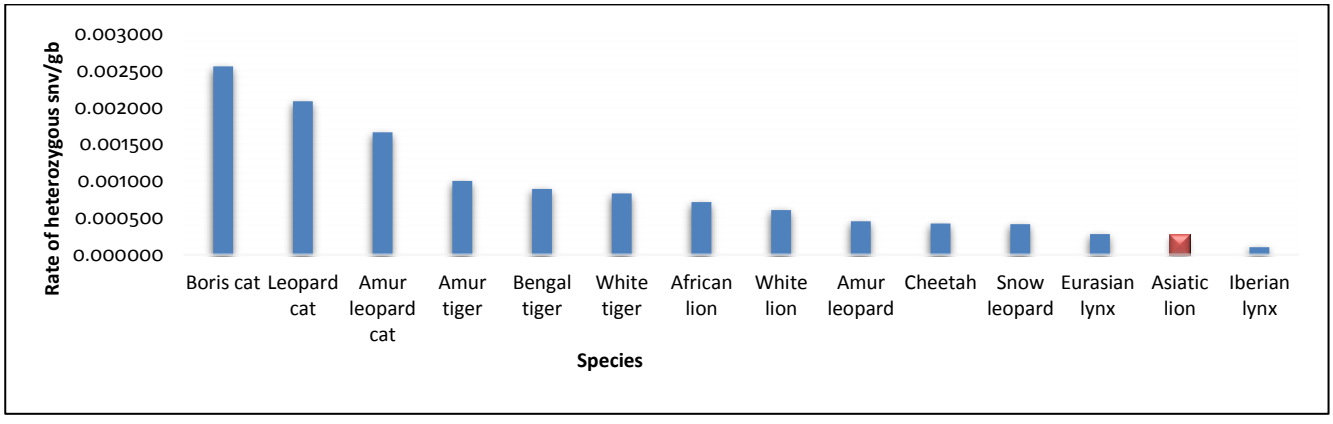


Fig. 2: Genomic diversity of Asiatic lion. The rate of heterozygous SNVs per base pair of genome is shown as an index of genomic diversity across felids.

d. Genome and gene evolution

To detect genome-wide structural variations (segmental duplications, intra- and inter-chromosomal rearrangements) the Asiatic lion genome was compared with that of the domestic cat. To identify changes that potentially underlie the morphological and physiological features unique to Asiatic lion, the genes and proteins in the Asiatic lion, domestic cat, Amur tiger, cheetah, giant panda, opossum, dog, mouse and humans were analyzed for orthology (Figure 3). The phylogenetic estimations obtained on the basis of these sequences were found to be coherent with previously published literature.

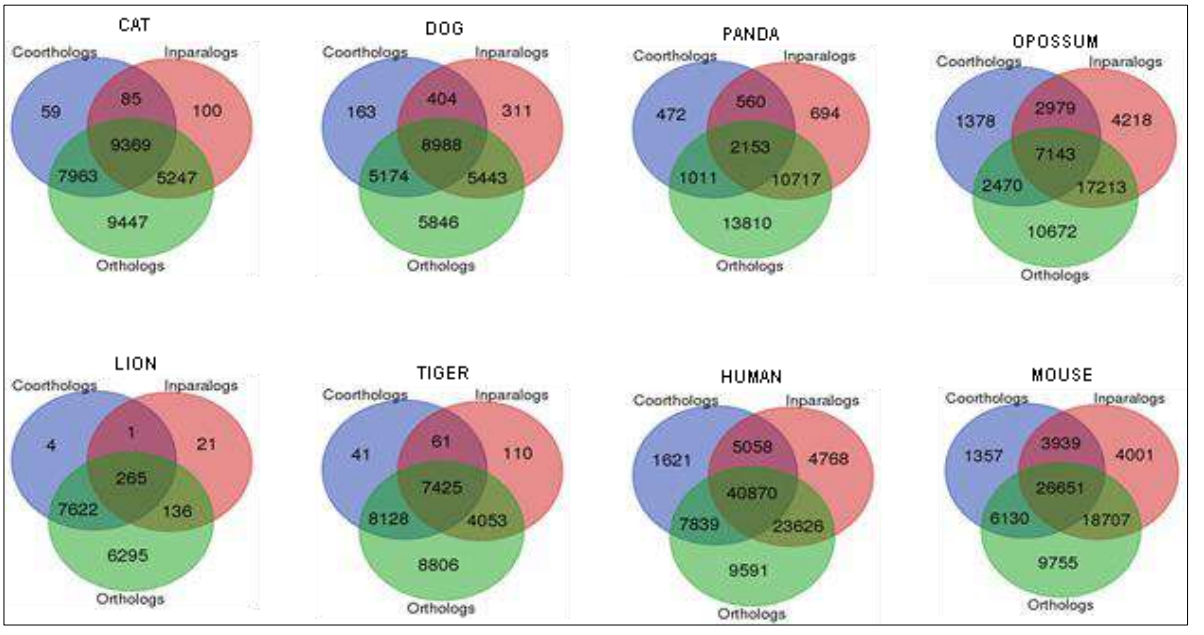


Fig. 3: Venn diagram showing orthologous protein families for eight species. Orthologs and coorthologsare result of speciation events and paralogs are the result of a duplication event within a genome.

Here, we have presented the first ever whole genome sequence of the Asiatic lion. Comparative analysis with other felids and mammalian genomes unravelled the evolutionary history of the Asiatic lion and its position among other felids. Findings on genome organization and evolutionary divergence analyses are concordant with those obtained for felid genomes in earlier complete genome studies. The low genomic variation we found in the Asiatic lion is typically reported for species with low population sizes and on the brink of extinction, which is important from the conservation point of view. Genomic and gene evolution findings in the study, especially those relevant to features characteristic of lions and felidsneed further corroboration through studies on multiple individuals before they can be used for conservation and management of the Asiatic lion.

SANDEEP GOEL

Testis Biology

Sandeep Goel

RESEARCH INTERESTS:

- Cryobiology of mammalian testicular tissue for fertility preservation
- Spermatogenesis and its augmentation for salvaging fertility

"The objective of our group is to understand the cryobiology of mammalian testis for the preservation of fertility and decipher the underlying biological mechanisms to minimize cryoinjury to cells and tissues. We are also interested in understanding the biological mechanisms underlying the process of spermatogenesis and the methods to augment it for salvaging fertility of endangered species."

Selected recent publications

- Goel S, Minami N (2019). Altered hormonal milieu and dysregulated protein expression can cause spermatogenic arrest in ectopic xenografted immature rat testis. *Scientific Reports* 9: 4036.
- Devi L, Pothana L, Goel S (2017). Dysregulation of angiogenesis-specific signaling in adult testis results in xenograft degeneration. *Scientific Reports* 7: 2605.
- Pothana L, Devi L, Goel S (2017). Cryopreservation of adult cervid testes. *Cryobiology* 74: 103-109.
- Pothana L, Venna NK, Devi L, Singh A, Chatterjee I, Goel S (2016). Cryopreservation of adult primate testes. *European Journal of Wildlife Research* 62: 619-626.
- Pothana L, Devi L, Venna NK, Pentakota N, Varma VP, Jose J, Goel S (2016). Replacement of serum with ocular fluid for cryopreservation of immature testes. *Cryobiology* 73: 356-366.

Spermatogenic arrest in xenografted testicular tissues: A hindrance in the preservation of fertility

Ectopic xenografting of the testis is a feasible option for preservation of male fertility and for understanding the mechanism of spermatogenesis and testicular maturation. This technique has been used for the production of mature gametes by grafting small pieces of testis tissue under the dorsal skin of immunodeficient mice recipients. Xenografting, complemented with cryopreservation of testis tissue, can find application in fertility preservation in children with malignancies who receive gonadotoxic treatment. It is also a useful strategy for conservation of endangered animals with high neonatal mortality rate. Production of live offspring from cryopreserved-xenografted testis of rabbit, and more recently from pig, showed the applicability of these techniques. Of the 23 species of mammals used as donors for testicular tissue xenografting to date, 15 showed complete spermatogenesis. In the remaining 7 species which primarily included endangered species such as Banteng, Mohor gazelle, Cuvier's gazelle (endangered ungulates), Iberian lynx (an endangered feline), common marmoset, laboratory rat, and humans, the spermatogenic arrest occurred at the spermatogonia, spermatocyte, or round spermatid stages. We recently showed that spermatogenesis was incomplete and arrested at the spermatocyte stage in xenografted testis from an endangered ungulate (Indian spotted mouse deer). It is known that genetics, hormonal, thermal, and toxic factors are implicated in spermatogenic arrest in humans. Although exogenous gonadotropin treatment of the recipient mice was demonstrated to aid completion of spermatogenesis in the xenografted testis of certain species, these results were inconsistent. Therefore, the key factors that lead to spermatogenic arrest in xenografted testis still remain unclear, and the underlying mechanism needs to be investigated to increase the efficiency of xenografting. Further insights into spermatogenic arrest in xenografts would help in developing methods to overcome it.

Spermatogenesis and fertility are dependent upon paracrine interactions between the somatic cells and the germ cells, and endocrine support from the pituitary gland. Development and differentiation of germ cells requires the interaction of germ cells with the supporting Sertoli cells in the epithelium of the seminiferous tubules. In situ, germ and somatic cells communicate closely in the testis for the progression of spermatogenesis (Fig. 1). An optimal hormonal milieu along with the expression of cell-specific proteins, and transcription regulators creates an intricate balance which regulates testicular growth and maturation, and germ cell proliferation and differentiation to enable the production of fertilization-competent gametes. Failure of germ cell-somatic cell interactions could be one of the causes of spermatogenic arrest in xenografted testis. Because rat testis xenografts show spermatogenic arrest, they can, therefore, serve as a suitable model for studying spermatogenic arrest. The purpose of the present study was to identify the factors that play a role in spermatogenic arrest using the rat-to-mouse testis xenograft model by evaluating endocrine changes in the recipients and changes in protein expression in xenografts.

Testes from immature rat pups were xenografted subcutaneously onto castrated adult immunodeficient mice recipients. Following xenografting, immature rat testis showed a progressive increase in weight over 8 weeks, suggestive of consistent growth of seminiferous tubules and supporting cellular structures and establishment of an effective vascular connection between the xenograft and the recipient, resulting in its survival. Histological evaluation of the xenografts collected at 8-week post-grafting showed the presence of pachytene-stage spermatocytes as the most advanced germ cells (Fig. 2), which suggests establishment of a synchronized hormonal interaction between the recipient's pituitary and the xenografted immature rat testis that induced spermatogenesis. However, the absence of elongated spermatids in the xenografted testis indicated that the spermatogenesis remained incomplete. Serum hormone analysis of recipients revealed that although the levels of serum luteinizing hormone (LH) and testosterone were normal in recipient mice, those of follicle stimulating hormone (FSH) were significantly high. The expression of FSH receptor (FSHR) decreased significantly in xenografts at 2-wk and was absent at 4- and 8-wk post-grafting (Fig. 3). Contrary to FSHR, LH receptor (LHR) was expressed in xenografts at all collection

time points but remained lower than that in donor and in situ control testis. Although elevated expression of androgen receptor (AR) was evident in donor testis, expression of AR in xenografts was not different from that in the in situ control testis post-grafting. Loss of FSHR from the xenografts could be attributed to sustained high serum FSH concentration in the recipients or to a higher sensitivity of rat Sertoli cells to elevated FSH level in castrated recipients. Interestingly, loss of FSHR from the xenografts did not seem to impair induction of spermatogenesis but likely prevented its completion. The xenografts also demonstrated the dysregulated expression of Sertoli cell-transcriptional regulators (WT1 and SOX9) and secretory proteins (SCF and GDNF).

In conclusion, results from our study suggest that an altered hormonal milieu in recipients and dysregulated protein expression in xenografts could be a potential cause of spermatogenic arrest in xenografted immature rat testis. Further investigations are warranted to analyse precise cellular composition of xenografts and decipher interactions between germ and somatic cells to further understand spermatogenic arrest in xenografted testis.

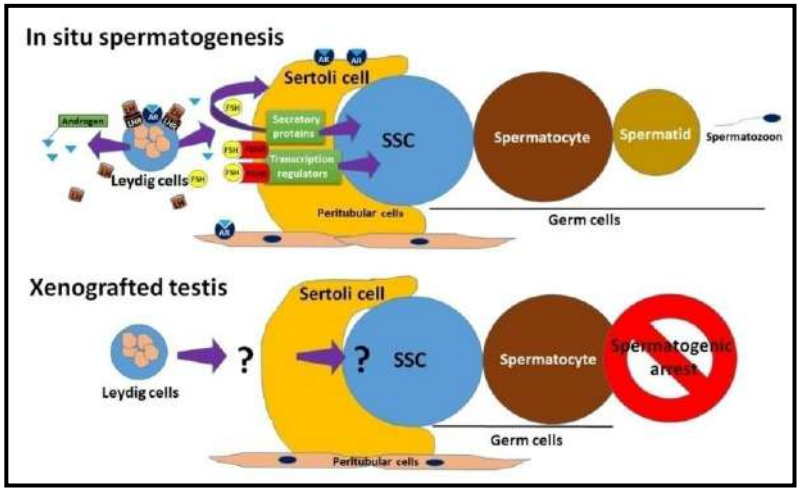


Fig. 1: A model for spermatogenic arrest in xenografted testis. In situ, germ and somatic cells communicate closely in the testis for the progression of spermatogenesis. An optimal hormonal milieu along with the expression of cell-specific proteins and transcription regulators create an intricate balance, which regulates germ cell proliferation and differentiation to enable the production of fertilization-competent gametes. In xenografted testis, the spermatogenesis is arrested. The question is what happens in xenografted testis which causes spermatogenic arrest? What are the critical factors (hormonal, cellular and molecular) which causes spermatogenic arrest in xenografts?

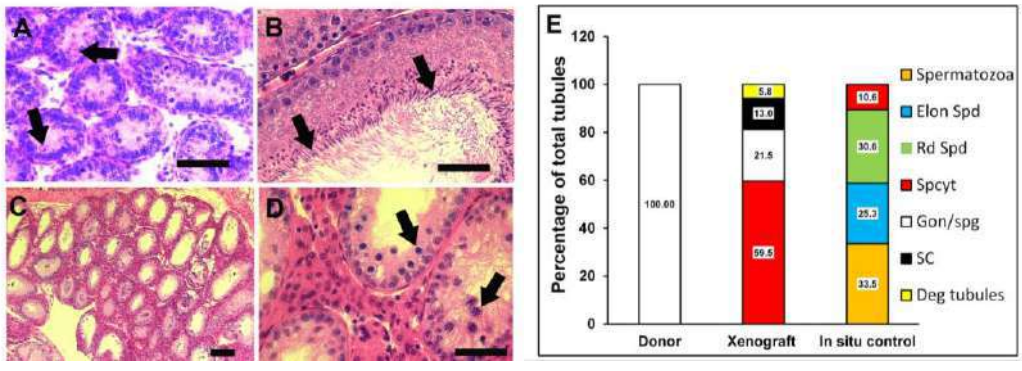


Fig. 2: Histological examination and quantitative assessment of seminiferous tubules for the most advanced germ cell type. (A) Immature donor tissue from a 7-day-old rat. Note that the most advanced germ cells at this age were gonocytes/spermatogonia (arrows). (B) A 9-wk-old in situ control testis. Note that the most advanced germ cells identified were spermatozoa. (C) Low and (D) high magnification image of a xenograft collected at 8-wk post-grafting. Note that the most advanced germ cells identified in the xenografts were pachytene-stage spermatocytes (D, arrows). (E) Percentage of seminiferous tubules with the most advanced germ cell type. Deg tubules, degenerated tubules; SC, Sertoli cell-only; Gon/spg, gonocytes or spermatogonia; Spcyt, pachytene spermatocytes; Rd Spd, round spermatid; Elon Spd, elongated spermatid; Spermatozoa, spermatozoa. Scale bar = 50 µm.

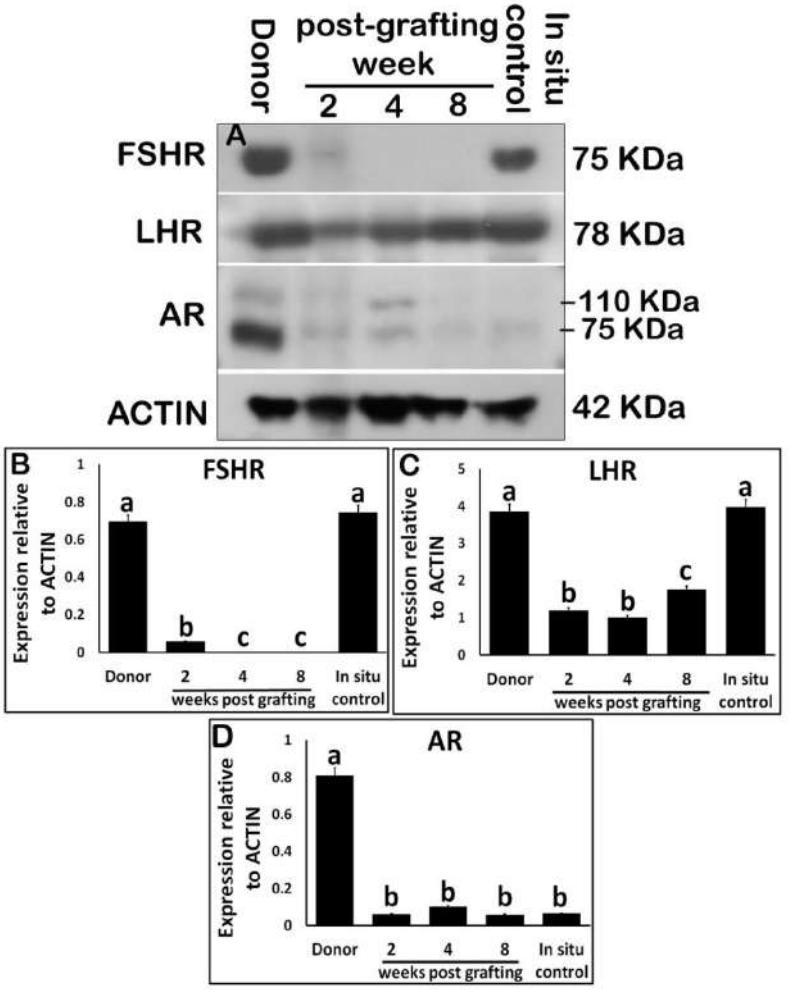


Fig. 3: Western blot analysis of xenografted testis at 2-, 4-, and 8-wk post-grafting for expression of hormone receptors. Protein expression in 7-day-old donor testes (donor) before grafting is presented as starting material and 9-week-old rat testis as in situ control. (A) Representative blot and densitometry analysis of (B) FSHR, (C) LHR, and (D) AR protein. Y-axis represents the intensity of bands relative to ACTB. Data are presented as mean ± SEM. Bars with different letters are significantly different at $P < 0.05$.

KRISHNAN H HARSHAN

Host-Virus Interactions: Molecular Perspectives

From left to right: Dibyashree, Dhiviya Vedagiri, Anjana CK, Divya Gupta, Poonam Manral, Haripriya Parthasarathy, Farsana SM (Front Row) Mohan Singh Moodu, Krishnan H Harshan, Soubhik Jana (Back Row)

RESEARCH INTERESTS:

- Regulation of antiviral pathways in innate immunity against viruses
- Understanding the regulatory mechanisms that provide the preferential advantage to viral translation

"The focus of our laboratory is the host-response to infections by human virus such as Dengue and Hepatitis C viruses. Through our studies we aim to characterize novel mechanisms of antiviral responses that regulate viral replication, translation and packaging"

Selected recent publications

- George A, Panda S, Kudmulwar D, Chhatbar SD, Nayak SC, Krishnan HH (2012). Hepatitis C virus NS5A binds to the mRNA Cap binding eIF4F complex and upregulates host translation initiation machinery through 4EBP1 inactivation. *Journal of Biol*ogical *Chemistry* 287: 5042-58.
- Panda S, Vedagiri D, Viveka TS, Jonddula RC, Krishnan HH (2014). Hepatitis CVirus Protein NS5A Coordinates a Unique Phosphorylation Dependent eIF4E Assembly on 40SRibosomes That Regulates IRES Activity. *Biochemical Journal* 462: 291-302.

Viruses are intracellular parasites that obligate living cells for their replication. In the process of their sustenance in the foreign host, viruses jeopardize the biological equilibrium of the host cell or organism. The outcome is the compromised survival of the infected cells that often leads to their death. On the other hand, several viruses have evolved to co-exist with the host that often results in life long relationship without causing major threats to the host.

Viruses form diverse groups based on the nature of genetic material, its polarity, presence of envelope etc. Interestingly, a significant fraction of the fatal or potentially fatal human viruses are RNA viruses. This is no different for the emerging or reemerging viruses. Examples include Dengue (DENV), Zika, West Nile, Japanese Encephalitis (JEV) and Influenza viruses. These viruses have single stranded RNA as the genetic material that is either positively or negatively polarized. Replication of the genetic material is critical for the generation of infectious virions. (+) stranded RNA genomes replicate to form (-) strand, which is further replicated to form several (+) stranded RNA that can either package into virions or can translate to synthesize viral proteins. (-) stranded genomes need to replicate to (+) strand and further to form (-) strand genomic RNA. One of the most critical steps for the viruses is to take control of the protein translation machinery in order to ensure the production of enough viral proteins. Running into several hundred in number, the translation apparatus cannot be encoded by viruses that package very small genomes, often limited to a few thousand bases. Different viruses engage with the translation machinery differently. Some of them totally hijack the translation apparatus while others manipulate the activity of various components. Irrespective of the various approaches the viruses take, it is evident that viruses distinctly modulate translation apparatus in ways that suit their needs. Therefore, translation is a step that viruses invest a lot in for regulation and hence presents itself as a critical one in effectively controlling the infection.

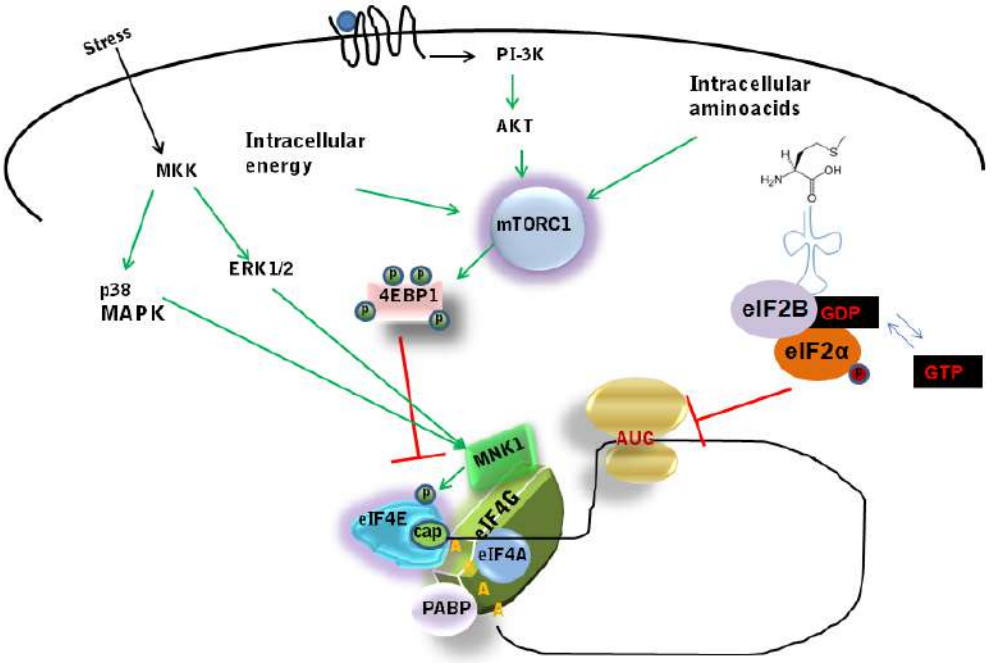


Figure 1. Regulation of protein translation initiation in eukaryotes

Initiation of translation is the major rate limiting step in protein synthesis in eukaryotes. Initiation is understood to be regulated by (i) limiting the availability of the cap-binding protein eIF4E, (ii) limiting its activity through modifications and (iii) limiting the recycling of ternary complex that delivers the initiator tRNAMet^{tRNA}. Various viruses have devised diverse strategies in enforcing the arrest of

host translation while finding a way for its own translation. Poliovirus cleaves a key cap-binding protein eIF4G thereby shutting down the host translation while continuing to translate its proteins using cap-independent mechanism through internal ribosome entry site (IRES) located in its 5'UTR. Hepatitis C Virus (HCV), on the other hand does not suppress host translation while promoting its own translation using IRES. Other viruses such as DENV use cap-dependent mechanisms that seem to be distinct from those used by the host mRNAs. Therefore, understanding the fine details of the requirements of viral translation is a pre-requisite in designing means to control viral propagation.

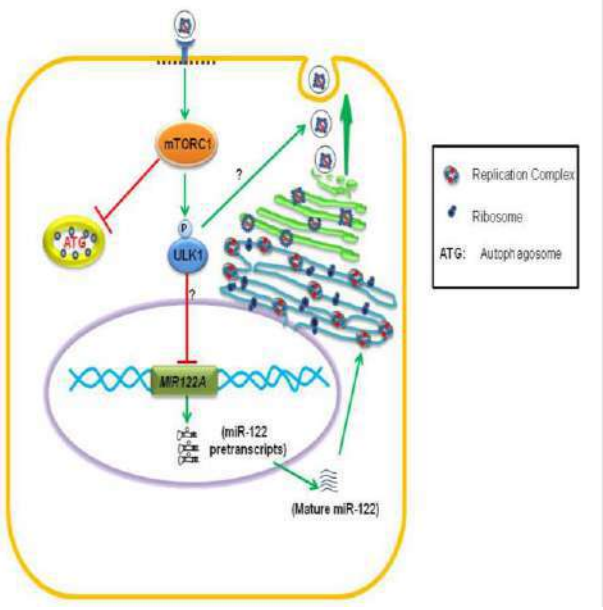


Figure 2. mTORC1 activated by HCV infection has anti-HCV functions

Our laboratory has been investigating the modulation of signal pathways that regulate host translation during infection by HCV, DENV and JEV. Some of these viruses inhibit host translation. We try to understand how viral translation is preferred or salvaged under conditions of severe suppression of host translation. mRNAs have unique regulatory elements in their 5' and 3' UTRs that assist in specific regulation of translation. We employ techniques such as polysome profiling and ribosome profiling in order to identify transcripts that are specifically regulated during viral infection. Proteins products of such transcripts could be critical in supporting viral translation. Our earlier studies revealed that HCV protein NS5A activates host translation and interacts with 40S ribosomes through a unique interaction mediated through eIF4E. In a recent study, we identified that mTORC1, a major regulatory complex of protein translation, antagonizes HCV replication through regulation of miR-122, a critical miRNA required for HCV replication (Figure 2).

RNA viruses predominantly inhabit the cytosol of the infected cells. Cytosolic membrane compartments are critical sites for viral replication. RNA viruses replicating in cytosol induce extensive structural and functional modifications on ER and promote formation of membrane vesicles that are exclusive units of viral replication factories. Host cells have devised mechanisms to identify the foreign RNA of viral origin. They are detected by endocytic or cytosolic receptors through recognizing pathogen associated molecular patterns (PAMPs). RIG-I and MDA-5 are two such receptors (RLRs, RIG-I like receptors) that bind to double stranded RNA replication intermediates and turn on a cascade of signaling events eventually activating interferon regulator factors (IRFs) that induce IFN production. Recent remarkable studies revealed that the access of entry into these vesicles is tightly regulated through nucleopore like structures through which RLRs are prevented from entering the replication factories. Our laboratory investigates the role of certain host factors that regulate the activity of these antiviral pathways.

K THANGARAJ

Evolutionary and Medical Genetics

From Left to Right: Rajan Jha, Jaydeep A, Sagnik Dhar, D.V.S. Sudhakar, Sunil K. Tripathi, Haneef (Front) Lomous Kumar, V. Purushotham, Narmada Ganapathy, G. Mala, Nipa Basak, Pratheusa Machha, Deepa Selvi Rani, K. Thangaraj

RESEARCH INTERESTS:

- Origin and affinities of modern human
- Genetic basis of
  - *Male infertility*
  - *Sex determination and differentiation*
  - *Mitochondrial disorders*
  - *Cardiovascular diseases*
- Ancient DNA
- Forensic genetics

"Our genomic study on skeletons from Roopkund Lake in Himalaya revealed that the bones belong to three genetically distinct groups - South Asian, Mediterranean and Southeast Asian - that were deposited during multiple events, separated in time by approximately 1000 years"

Selected recent publications

- Harney E, Nayak A, Patterson N, JoglekarJ, Mushrif-Tripathy V, Mallick S, Rohland N, Sedig J, Adamski N, Bernardos R, Broomandkhosh bacht N, Culleton BJ, Ferry M, Harper TK, Michel M, Oppenheimer J, Stewardson K, Zhang Z, Harashawaradhana[11], Bartwal MS, Kumar S, Diyundi SC, Roberts P, Boivin N, Kennett DJ, Thangaraj K, Reich D, Rai N (2019). Ancient DNA from the skeletons of Roopkund Lake reveals Mediterranean migrants in India. *Nature Communications* 10: 3670.
- Rani DS, Rajender S, Pavani K, Chaubey G, Rasalkar AA, Gupta NJ, Deendayal M, Chakravarty B, Thangaraj K (2019). High frequencies of Non Allelic Homologous Recombination (NAHR) events at the AZF loci and male infertility risk in Indian men. *Scientific Reports* 9: 6276.
- Tamang R, Chaubey G, Nandan A, GovindarajP, Singh VK, Rai N, Mallick CB, Sharma V, Sharma VK, Shah AM, Lalremruata A, Reddy AG, Rani DS, Doviah P, Negi N, Hadid Y, Pande V, Vishnupriya S, van Driem G, Behar DM, Sharma T, Singh L, Villems R, Thangaraj K (2018). Reconstructing the demographic history of the Himalayan and adjoining populations. *Human Genetics* 137: 129-139.

- Nakatsuka N, Moorjani P, Rai N, Sarkar B, Tandon A, Patterson N, Bhavani GS, Girisha KM, Mustak MS, Srinivasan S, Kaushik A, Vahab SA, Jagadeesh SM, Satyamoorthy K, Singh L, Reich D, Thangaraj K (2017). The promise of discovering population-specific disease-associated genes in South Asia. *Nature Genetics* 49: 1403-1407.
- Chaubey G, Ayub Q, Rai N, Prakash S, Mushrif-Tripathy V, Mezzavilla M, Pathak AK, Tamang R, Firasat S, Reidla M, Karmin M, Rani DS, Reddy AG, Parik J, Metspalu E, Rootsi S, Dalal K, Khaliq S, Mehdi SQ, Singh L, Metspalu M, Kivisild T, Tyler-Smith C, Villems R, Thangaraj K (2017). "Like sugar in milk": reconstructing the genetic history of the Parsi population. *Genome Biology* 18: 110.

Our primary research interest is to understand the origin and affinities of modern humans, and the genetic basis of diseases. We have demonstrated that the Indian populations are the descendents of the very first modern human that migrated Out-of-Africa about 60,000 years ago. Since then most of the Indian populations are in isolation and practicing endogamy, at least for the last 2000 years. As a result, Indian populations acquired a unique set of genetic variations, which are of Indian-specific and responsible for causing population-specific diseases in India. Therefore, we have been assessing the genetic variations among Indian populations to understand their origin, health and disease.

Ancient DNA from the skeletons of Roopkund Lake reveals Mediterranean migrants in India

Nestled deep in the Himalayan Mountains at 5029m above sea level, Roopkund Lake is a small body of water (~40min diameter) that is colloquially referred to as Skeleton Lake due to the remains of several hundred ancient humans scattered around its shores (Fig. 1). Little is known about the origin of these skeletons, as they have never been subjected to systematic anthropological or archaeological scrutiny, in part due to the disturbed nature of the site, which is frequently affected by rockslides, and which is often visited by local pilgrims and hikers who have manipulated the skeletons and removed many of the artifacts. There have been multiple proposals to explain the origins of these skeletons. Local folklore describes a pilgrimage to the nearby shrine of the mountain goddess, Nanda Devi, undertaken by a king and queen and their many attendants, who - due to their inappropriate, celebratory behavior - were struckdown by the wrath of Nanda Devi. It has also been suggested that these are the remains of an army or group of merchants who were caught in a storm. Finally, it has been suggested that they were the victims of an epidemic.

To shed light on the origin of the skeletons of Roopkund, we analyzed their remains using a series of bioarcheological analyses, including ancient DNA, stable isotope dietary reconstruction, radiocarbon dating, and osteological analysis. Genome-wide ancient DNA analysis for 38 skeletons from Roopkund Lake, revealed three distinct groups. A group of 23 individuals have ancestry that falls within the range of variation of present-day South Asians. A further 14 have ancestry typical of the eastern Mediterranean. We also identified one individual with Southeast Asian-related ancestry. Radiocarbon dating indicates that these remains were not deposited simultaneously. Instead, all of the individuals with South Asian-related ancestry date to ~800 CE (but with evidence of being deposited in more than one event), while all other individuals date to ~1800 CE. These differences are also reflected in stable isotope measurements, which reveal a distinct dietary profile for the two main groups.

High frequencies of Non Allelic Homologous Recombination (NAHR) events at the AZF loci and male infertility risk in Indian men

Deletions in the AZoospermia Factor (AZF) regions (spermatogenesis loci) on the human Y chromosome are reported as one of the most common causes of severe testiculopathy and spermatogenic defects leading to male infertility, yet not much data is available for Indian infertile men. Therefore, we screened for AZF region deletions in 973 infertile men consisting of 771 azoospermia, 105 oligozoospermia and 97 oligoteratozoospermia cases, along with 587 fertile normozoospermic men. The deletion screening was carried out using AZF-specific markers: STSs (Sequence Tagged Sites), SNVs (Single Nucleotide Variations), PCR-RFLP (Polymerase Chain Reaction

- Restriction Fragment Length Polymorphism) analysis of STS amplicons, DNA sequencing and Southern hybridization techniques. Our study revealed deletion events in a total of 29.4% of infertile Indian men. Of these, non-allelichomologous recombination (NAHR) events accounted for 25.8%, which included 3.5% AZFb deletions, 2.3% AZFbc deletions, 6.9% complete AZFc deletions, and 13.1% partial AZFc deletions. We observed 3.2% AZFa deletions and a rare long AZFabc region deletion in 0.5% azoospermic men (Fig. 2). This study illustrates how the ethnicity, endogamy and long-time geographical isolation of Indian populations might have played a major role in the high frequencies of deletion events.

Novel Variations in β-Myosin Heavy-Chain Gene (β -MYH7) and Its Association in South Indian Women with Cardiomyopathies

South Asia has the highest proportion of cardiovascular diseases (CVDs), including heart attacks and strokes of anyregion globally, probably due to their lifestyle and genetic architecture. Cardiomyopathy is one of the CVDs, which changes the heart morphology and damages the heart pumpingability, and it consequently leads to heart failure and sudden cardiac arrest in all age groups, particularly in young children, adults, and competitive athletes. Two major types of cardiomyopathies are dilated cardiomyopathy (DCM) and hypertrophic cardiomyopathy (HCM), each of which has significant heritable components. HCM is identified with left ventricular increased wall thickness, mostly the involvement of interventricular septum, myocardial fibrosis, myocyte disarray, and affects diastolic function. Although debated, the estimated occurrence of HCM is approximately 0.2% or 1:500. DCM is detected with left ventricular dilatation, myocardialfibrosis, systolic dysfunction, myocyte death, and expected occurrence of approximately 1:2500. Clinical heterogeneity varies from asymptomatic to symptomatic that results in failure of heart and sudden heart attack. Heterogeneity occurs because of environmental impacts such as food habits and exercise or genetic impacts due to modifying effects of genetic materials, or the existence of more than one disease-causing mutations in patients. It has been well established that a mutation in sarcomeric genes can cause cardiomyopathies. In addition, compound mutations were also reported in a few HCM cases in association with early disease onset. Patients with double and triple mutations displayed severe clinical symptoms because of the compound effect. Yet the molecular mechanisms for SNPs (single nucleotide polymorphisms) that cause these diseases have not been fully understood. We have sequenced all exons and their flanking regions of the *β-MYH7* gene in 188 Indian women consisting of 33 hypertrophic cardiomyopathy (HCM), 48 dilated cardiomyopathy (DCM), and 107 healthy controls. Our study showed 21 variations in *β-MYH7* gene, including 7 novel mutations. In addition, we compared this dataset with our previously studied datasets of seven other sarcomere genes (*ACTC, TNNT2, MYL2, MYBPC3, TPM1, TNNI3,* and *MYL3*) and found no causative mutation, confirming the nonexistence of compound heterozygosity. Interestingly, we detected a Val431Met mutation exclusively in patients, and its pathogenicity has been predicted using the protein homology model. In the native protein, Val431 is evolutionarily conserved across many species. In the homology model, mutant Met431 gets further buried in the hydrophobic core by creating an aberranthydrophobic interaction with Leu352. As a result, it probably reduces the spatial distances between other hydrophobic interactions in the hydrophobic core that may produce steric hindrance and strain. It may lead to deviation in the structure (root meanssquare deviation [RMSD] of ~3.9), and might possibly causing the cardiac remodelingand cardiomyopathy.

Homozygous R627W mutations in *POLG* cause mitochondrial DNA depletion leading to encephalopathy, seizures and stroke-like episodes

Mitochondrial disorders are complex genetic diseases, caused by mutations in either mitochondrial DNA (mtDNA) or nuclear encoded genes that exhibit remarkable phenotypic heterogeneity with variableage of onset. More than

99% of mitochondrial proteins required for maintaining the structure, function and stability of mtDNA molecules are encoded by nuclear genes. Mitochondrial DNA polymerase gamma (pol γ) is a nuclear-encodedprotein found in mitochondria and is essential for maintaining the integrity of the mitochondrial genome during replication and repair. The holoenzyme of human pol γ comprises a catalytic subunit of 140 kDa (encoded by *POLG*) and a homodimeric accessory subunit (encoded by*POLG2*). Mutations in *POLG* are a major cause of mitochondrial disease that result in the depletion and/or accumulation of multiple deletions of mtDNA. We analysed four South Asian patients from three unrelated families with multisystem mitochondrial disease. We identified homozygous *POLG* c.1879C>T; p.R627W mutations in two siblings from a consanguineous South Asian family (Fig. 3) following targeted resequencing of 75 nuclear-encoded mitochondrial genes. Both patients presented with encephalopathy, seizures and stroke-like episodes, and mitochondrial DNA depletion was confirmed in the proband's muscle tissue. Subsequent Sanger sequencing of *POLG* in a further 275 unrelated probands with genetically unconfirmed mitochondrial disease revealed a third unrelated proband with a similar phenotype harboring homozygous c.1879C>T; p.R627W mutations and a fourth patient, with amilder clinical disorder, harboring compound heterozygous *POLG* c.1879C>T; p.R627W and c.2341G>A; p.A781T mutations. Given the endogamous practices in the Indian subcontinent, the homozygous *POLG* c.1879C>T; p.R627W mutations should be excluded in South Asian patients presenting with encephalopathy, seizures and stroke-like episodes.

ARVIND KUMAR

Understanding dysregulation of transcription regulatory mechanisms underlying altered neural circuitry in mouse model of depression

From Left to Right: Arvind Kumar, Thasneem Musthafa, Shams-ul-Haq, Sachin Singh, R. Gajendra Reddy, Unis Ahmad Bhat, Harish Iyer, Annapoorna PK, Bedaballi Dey, Niharika Awasthi

RESEARCH INTERESTS:

- Uncovering molecular mechanisms in aetiology of complex neuropsychiatric disorders such as addiction, depression and comorbid cognitive disorders.
- Histone based epigenetic & transcription regulatory mechanisms
- Non-coding RNA based epigenetic & transcription regulatory mechanisms

"Our lab is involved in uncovering the histone based and non-coding RNA based transcription regulatory mechanisms in aetiology of complex neuropsychiatric disorders such as addiction, depression and comorbid cognitive disorders"

Selected recent publications

- Khandelwal N, Dey S, Chakravarty S, Kumar A (2019). MiR-30 family miRNAs mediate the effect of chronic social defeat stress on hippocampal neurogenesis in mouse depression model (Manuscript in Revision in *Frontier in Molecular Neuroscience*)
- Leighton LJ, Wei W, Marshall PR, Ratnu VS, Li X, Zajaczkowski EL, Spadaro PA, Khandelwal N, Kumar A, Bredy TW (2019). Disrupting the hippocampal Piwi pathway enhances contextual fear memory in mice. *Neurobiology of Learning and Memory* 161: 202-209.
- Ghosh S, Sinha JK, Khandelwal N, Chakravarty S, Kumar A, Manchala R (2018). Increased stress and altered expression of histone modifying enzymes in brain is associated with aberrant behaviour in vitamin B12 deficient female mice. *Nutritional Neuroscience* 25: 1-10.
- Pathak SS, maitra S, Chakravarty S, Kumar A (2017). Histone lysine demethylases of JMJD2 or KDM4 family are important epigenetic regulators in reward circuitry in the etiopathology of depression. *Neuropsycho pharmacology* 42: 854-863.

- Chakravarty S, Jhelum P, Bhat UA, Rajan WD, Maitra S, Pathak SS, Patel AB, Kumar A (2017). Insights into the epigenetic mechanisms involving histone lysine methylation and demethylation in ischemia induced damage and repair has therapeutic implication. *Biochimica et Biophysica Acta: Mol. Basis of Disease* 1863: 152–164.

Depression is a debilitating psychiatric disorder taking a heavy toll on individuals, families and societies throughout the globe. There are medications, but not very efficacious in nearly half of the affected population. The irony is a dearth of new therapeutics in last 5-6 decades; the reason being our poor understanding of the molecular mechanisms in aetiology. Therefore, better insight into the underlying mechanisms is warranted and our group is passionately pursuing research in this direction using mouse models of depression, and related affective disorders.

The focus in our group in the last few years has been on uncovering epigenetic and transcriptional regulatory mechanisms, which we have reported to be dysregulated in neural circuitries controlling emotions/mood and motivation. The genes/proteins involved inneuroglial responses to chronic perturbation of the nervous system by chronic or repeated stressful events are being studied in detail. This has aided us in gaining better mechanistic insights into neurogenic, neuroglial and neuroplastic changes associated with neuropathologies. This approach might help us not only in the discovery of molecular markers, but also in our endeavour to develop better and more efficacious therapeutics.

Dysregulation in miRNA synthesis pathway and miRNA-mRNA networks appear to be involved in altered hippocampal neurogenesis & neuroplasticity in mouse depression model

In the last few years, we have uncovered dysregulation in hundreds of non-coding RNAs including miRNAs, in the hippocampal neurogenic region dentate gyrus (DG). One of the nuclear proteins required for processing of primary miRNAs into precursor miRNAs, Drosha, was found attenuated in DG of mice that developed anhedonia, hallmarks of depression following 10 days of chronic social defeat stress (CSDS).As hypothesized, large number of miRNAs in DG were downregulated in defeated mice compared to control animals. In this CSDS paradigm, the C57bl/6 mouse is attacked by a larger, older, more aggressive CD1 mouse for 5 minutes each day and spends the next 24 hwith the aggressor separated by a perforated partition. Then, every day the C57 mouse has to undergo the same ordeal. Subsequently, when tested for anxiety and depression, most of the defeated animals show hyper-anxiety and anhedonia, indicating depression and related mood disorders. Our miRarray data analysis led us to identify hundreds of dysregulated miRNA species in the neurogenic region. Out of these altered miRNAs (many up and down regulated), dysregulation of miR-30 family was an exciting discovery. Most of the family members, miR30a, b, c, d and e were found downregulated in DG in depression.

DG neurogenesis in adult brain has been reported to decrease in animal models of depression, including the model (CSDS) we used. Even we have evidence of attenuated neurogenesis in the model we used in our studies, as evident by downregulation of nestin and doublecortin (Dcx), the neural stem or progenitor cell (NSCs/NPCs) proliferation and early neuronal differentiation markers, respectively. To study the role of miRNAs in NSCs/NPCs from DG, *in vitro* neurosphere cultures in proliferation and differentiation were grown and miRarrays were performed. Once again several differentially expressed miRNAs were observed and interestingly upregulation of miR-30 family miRNAs were observed upon differentiation. In the end, we performed gene (mRNA) arrays followed by bioinformatics analysis, miRNA manipulations in cell lines and luciferase assays, to identify the gene targets of miR-30 family miRNAs. Our data suggest that miR-30 family members mediate CSDS induced depression and related mood disorder phenotype in mice, altering hippocampal neurogenesis and neuroplasticity. The targets through which miR-30s does this are the epigenetic/transcription regulators *Mll3*/*Runx1,* respectively. The data have been deposited in NCBI's Gene Expression Omnibus and are accessible through GSE 132823

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE132823

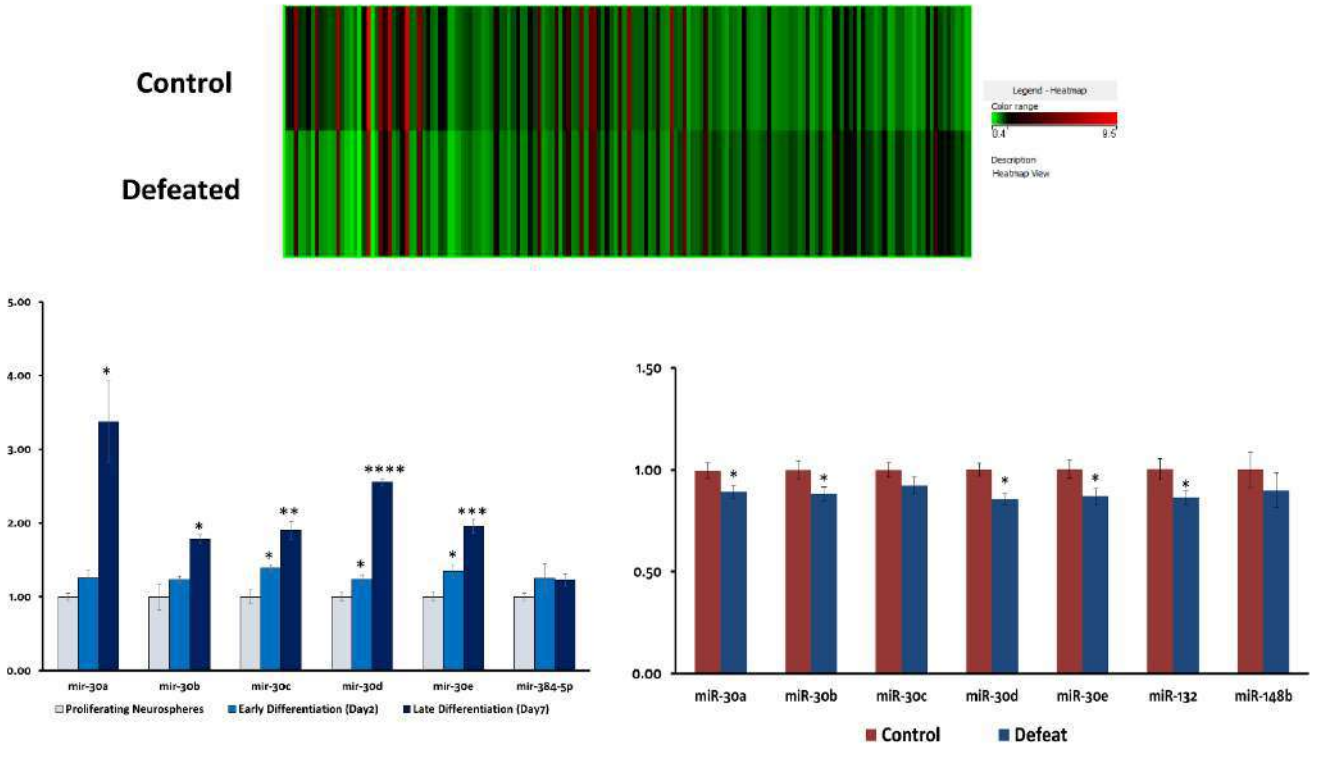


Fig. A) Snapshot of heat map depicting differentially regulated miRNAs in neurogenic DG of mice showing depression-like condition following repeated social defeat for 10-days; **B)** Validation experiment using RT-qPCR shows attenuation in the transcript levels of miR-30 family members in DG samples from defeated mice compared to control mice; **C)** shows the role of miR-30 family members in proliferation and differentiation of DG neural stem or progenitor cells.

LEKHA DINESH KUMAR

Role of *Wnt* Signalling in EMT and Development of Colon Cancer

From left to right: Aparna Golani, Khushboo Kourani, Aviral Kumar, Lekha Dinesh Kumar, Surabhi Kumari, Yamini Meshram

RESEARCH INTERESTS:

- Biomarker discovery for various cancers
- Biological drugs and targeted delivery
- Natural products as anticancer drug
- Transgenic murine models
- Mechanism of drug resistance

"Since early diagnosis is the key for cure in the case of cancers, our lab is involved in biomarker discovery for various cancers. Another activity in our lab is discovery of biological drugs and targets which will be a replacement for chemotherapy"

Selected recent publications

- Chakraborty S, et. al., (2019). Vimentin activation in early apoptotic cancer cells errands survival pathways during DNA damage inducer CPT treatment in colon carcinoma model. *Cell Death and Disease* 10: 467.
- Dinesh Kumar L. (eds) In: RNA Interference and Cancer Therapy. Methods in Molecular Biology, Springer Protocols (2019). Vol 1974. Humana, New York, NY. doi: https://doi.org/10.1007/978-1-4939-9220-1_19
- Beevi SS, et. al., (2019). Biodrug Suppresses Breast and Colorectal Cancer in Murine Models. RNA Interference and Cancer Therapy. Methods in Molecular Biology, Springer Protocols Vol 1974. Humana, New York, NY. doi: https://doi.org/10.1007/978-1-4939-9220-1_19
- Katoch A, et. Al., (2018). Dual role of Par-4 in abrogation of EMT and switching on Mesenchymal to Epithelial Transition (MET) in metastatic pancreatic cancer cells. Mol. Carcinog. Apr 19. doi: 10.1002/mc.22828. [Epub ahead of print] PMID:29672923
- Nayak D, et. al., (2017). Inhibition of Twist1-mediated invasion by Chk2 promotes premature senescence in p53-defective cancer cells. *Cell Death & Differentiation* 24: 1275-1287.

Patents Granted

Lekha Dinesh Kumar, Vinod Kumar Verma, Rekha A Nair, Jem Prabhakar and Jayasree Kattoor. Biomarkers useful for detection of grades of human breast cancer. Indian Patent No.313602, Date of Publication, 31.05.2019

Though chemotherapy is one of the key strategies of cancer treatment, a major problem faced by clinicians in the management of the disease is that of drug resistance. A majority of drug development programs in cancer aim at selective elimination of cancer cells by modulating apoptotic mechanisms. Families of drugs which damage DNA and cause hindrance to its repair mechanisms are widely used in cancer therapeutics. Epithelial to mesenchymal transition (EMT) is one of the earliest events in cancer cell metastasis. EMT can also be viewed as a preparatory mechanism for the epithelial cells to gain motility, and attain mesenchymal shape which results in alteration of various cellular cytoskeletal structures. The mechanism by which EMT facilitates cells to achieve drug resistance is in its very initial phase of research. Whether EMT orchestrates a collateral event during drug resistance or the initiation of EMT plays a leading role in resistance by creating an escape strategy for the cancer cells to release themselves from the stressed environment is not yet clear. Out of all EMT factors, Vimentin is the most important facilitator for mesenchymal cells for their migration and invasion. Apart from its cytoskeletal role, Vimentin functions as a signaling protein during EMT cascade. Thus, induction of Vimentin during EMT cascade and role of Vimentin to confer survival responses bypassing apoptotic progression during therapeutic intervention is a real challenge to cancer biologists. Extensive research over the past decades has unfolded several aspects of EMT, but little is known about how cancer cells survive by activating EMT for circumventing apoptosis/anoikis. The typical role of EMT in drug resistance and stemness acquisition in cancer cells is beginning to be understood only recently.

Majority of chemotherapeutic drugs induce DNA damage response (DDR) signaling cascades leading to cell death. However, dysregulation of DDR pathways might result in hypersensitivity or resistance of tumours to therapy. Identification of the molecular mechanisms underlying this phenomenon is essential for enhancing the potential of cancer therapy. The activation and stabilization of Snail by ATM kinase and its further involvement in promoting metastasis (when challenged by a DNA damaging agent) poses vital questions concerning the simultaneous progression of apoptosis and EMT in cancer cells. Although CPT, a potent topoisomerase-1 poison, activates apoptotic pathways within the cells having wild type p53 background, it is unclear why the DNA damage sensor ATM kinase activates Snail, a potential EMT inducing transcription factor. But these concepts of premature activation of pro metastatic and pro survival responses are rudimentary. To provide more concrete evidence, we tested this phenomenon in both *in vitro* (HCT-116, SW620, A549 cells) and *in vivo* (autochthonous murine colorectal carcinomas) models. Though CPT treatment triggers apoptotic progression, the simultaneous activation of EMT leading to survivability cannot be overlooked.

We provide evidence of stimulation of EMT factors in Apc knockout colorectal carcinoma model. Our results implied that CPT-treated Apc knock out cohorts depicted much extended and fused crypts, and villus and immunohistochemistry analyses of intestinal sections obtained from these CPT treated mice showed a significant rise in p^{ser38}Vimentin/Vimentin expression (Fig 1). The results demonstrate co-parallel incidence of EMT induction, as well as stimulation of early apoptotic factors, achieved in the same population of colon cancer cells undergoing CPT treatment. After a prolonged 36 h of CPT treatment, the late phase of apoptosis (caspase activation, nuclear condensation and formation of apoptotic bodies) was initiated, while a majority of the EMT and survival factors (NFκB, Survivin, p^{Ser38}Vimentin, Snail-1, β-catenin, c-FLIP) declined significantly at the 48 h time point. Blocking the EMT process by DIM (Di-indole methane) showed a profound shift of EMT towards apoptosis, suggesting the activation of the EMT cascade as a

result of CPT treatment. Based on the above evidence, we propose that apoptotic progression was delayed due to the activation of EMT which prompted a strong survival response within the cell (Fig.2). The early apoptotic signatures observed along with EMT could have triggered intense cross talks within the cellular survival machinery causing cells to escape cell death or aided them in further activating signaling to acquire drug resistance.

Both EMT and apoptosis were thought to be mutually exclusive fates involving distant machinery in a cell until recently. Our findings of sustained Vimentin expression in apoptotic cells even after 48 hrs of CPT treatment compared to viable cells supports the emerging concept of unification between these two evolutionarily conserved processes. The hypothesis is further supported by the fact that the viable cells that showed absence of Vimentin expression were also positive for Annexin V-mvenus immunostaining. These cells under stress release certain paracrine signals prompting anti-apoptotic or pro-survival responses which might have activated EMT factors initially in both the populations, but as time progressed the viable cells alone stimulated certain mechanisms that restored their epithelial integrity by mitigating the EMT crosstalk. However, the expression of Vimentin solely at 12 h time point within the viable population is undoubtedly a unique feature.

Majority of FDA approved drugs for cancer damage DNA and cause hindrance to its repair mechanisms leading to cell death. Nonetheless, our findings strongly implied that these DNA damaging agents not only elicited EMT phenotype but also elevated the migratory potential of both the epithelial cells (HCT-116 and A549). Though induction of apoptosis by DNA damaging agents is a normal event, the existence of EMT factors in these apoptotic cells hastened their invasiveness thus posing a serious question about the rationality of using DNA damaging drugs in cancer therapeutics. Since most of the EMT factors coincide with machinery involved in drug resistance, careful remodeling and screening of DNA damaging drugs are required to avoid activation of unwanted EMT factors. The present findings that a low dose of CPT was able to restore the normal colon crypt and villi structures in presence of DIM in Apc knock out colorectal carcinoma models suggest the use of combinational therapy for circumventing the aforementioned issues.

Vimentin helps in maintenance of mesenchymal structures and cellular motility and we have observed diminishing of the survival aspects of Vimentin by siRNA mediated silencing. Notably, we have shown that even FAS receptor trafficking is dependent on Vimentin since it controls the expression of c-FLIP. NFκB, one of the major transcription modulators of EMT, also showed concomitant increase along with Vimentin expression both *in vitro* and *in vivo*. Hence, the present study unveils a positive feedback loop between these two evolutionarily conserved proteins-Vimentin and NFκB. A recent report showed that NFκB binding sites on Vimentin promoter results in its activation with T-cell leukemia virus type 1 Tax protein.

ATM kinase is a major DNA damage sensor and upon activation it halts the cell cycle and stimulates effectors of DNA repair pathways. Additionally, activation of ATM kinase also triggers several cellular survival responses and EMT which can be detrimental for the patients undergoing chemotherapy leading to further drug resistance. In our study we also observed that ATM kinase plays a pivotal role in facilitating Vimentin to execute its functions related to hindering apoptosis as well as boosting invasion. Vimentin interacts with several kinases and gets phosphorylated at various sites depending on the type of interaction. Consequently, phosphorylated Vimentin plays an essential role in cellular motility and survival. Our work elucidates a novel signaling link between ATM kinase and vimentin as we have shown that both these proteins physically interact with each other and activate ATM kinase helping phosphorylation of Vimentin. Thus the anomalous activation of EMT and cellular survival pathways during initial phases of drug treatment observed in this study as well as by several other groups questions the basic concept of chemotherapeutic approach by DNA damaging agents. Furthermore, our results unveiled a novel link

between Vimentin and ATM signaling, orchestrated via binding interaction between Vimentin and ATM kinase. Finally, we observed a significant alteration of crypt-villus morphology upon combination of DIM (EMT inhibitor) with CPT for 48 h treatment and found that it nullified the background EMT signals thus improving the efficacy of the DNA damaging agent. Therefore there is an imminent need for designing novel therapeutic approaches for the tumor cells to achieve committed stages of apoptosis efficiently, thus eliminating the problems of drug resistance.

MEGHA KUMAR

Cell and Developmental Biology

From left to right: Kirti Prasad, Varsha R, Sulagna Mukherjee, Megha Kumar

RESEARCH INTERESTS:

- Mitosis during early embryonic development
- Mitotic aberrations and developmental disorders
- Zebrafish embryogenesis

"Mitosis is a fundamental process during embryogenesis and mitotic aberrations result in developmental disorders such as microcephaly, aneuploidy syndromes and embryonic lethality. We study the molecular mechanisms regulating mitosis to understand the basis of these developmental disorders using zebrafish embryo as the model system"

My group is studying the mitotic machinery and the associated signaling pathways involved in various steps of cell division, resulting in faithful separation of DNA and cytoplasm during early divisions in the zebrafish embryo. As a molecular handle to the complex mitotic machinery, we are currently investigating the role of Centrosomal protein family (CEPs) during cell division. Our preliminary results indicate that CEP genes are required for proper spindle orientation, resulting in correct specification of cell fate and normal tissue architecture during early embryonic development.

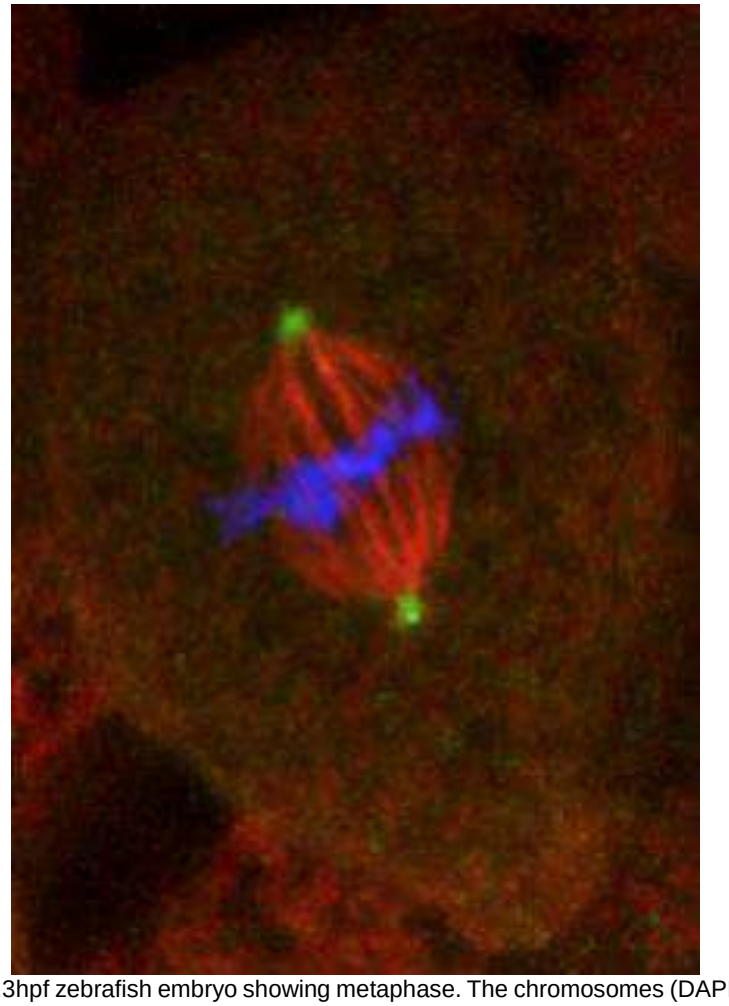


Fig. 1: Surface blastomere of 3.3hpf zebrafish embryo showing metaphase. The chromosomes (DAPI, blue) are tightly congressed in the centre, held by the spindle microtubules (α tubulin, red) and centrosomes (γ tubulin, green). The CEP proteins also localize to the centrosomes with γ tubulin.

SANTOSH KUMAR

Receptor Signalling and Immune Response

From left to right: Sitanshu Kumar Sarangi, Ketaki Bhagwat, Samrajya Lakshmi, Santosh Kumar, Rini Jacob

RESEARCH INTERESTS:

- Immunoreceptor signaling
- T cell responses in *Helicobacter pylori* infection

- **We wish to understand the principles of immunoreceptor signaling, using the tools of *in vitro* reconstitution, fluorescence imaging, and cellular biochemistry.**
- **We wish to understand the T cell and NK cell responses in human diseases, using the tools of single cell sequencing, genomics, and cellular biochemistry.**

Selected recent publications

- Kumar S, Jain S (2018). Immune signaling by supramolecular assemblies. *Immunology* 155: 435-445.
- Kumar S (2018). Natural killer cell cytotoxicity and its regulation by inhibitory receptors. *Immunology* 154: 383-393.
- Kumar S, Rajagopalan S, Sarkar P, Dorward DW, Peterson ME, Liao H, Guillermier C, Steinhauser ML, Vogel SS, & Long EO (2016). Zinc-induced polymerization of killer-cell Ig-like receptor into filaments promotes its inhibitory function at cytotoxic immunological synapses. *Molecular Cell* 62: 21-33.
- Kumar S, Sarkar P, Sim MJW, Rajagopalan S, Vogel SS, & Long EO (2015). A single amino acid change in inhibitory killer cell Ig-like receptor results in constitutive receptor self-association and phosphorylation. *The Journal of Immunology* 194: 817-826.

Transmembrane immune signaling

Ligand binding-induced dimerization of a receptor tyrosine kinase, which triggers the intrinsic kinase activity of the receptor, was a seminal discovery in transmembrane signaling. It now appears that dimerization or oligomerization is a general principle in transmembrane signaling. The HLA-C-specific inhibitory killer-cell Ig-like receptor (KIR) is expressed on the surface of human natural killer (NK) cells, and performs an important function of preventing NK cell cytotoxicity towards healthy cells. KIR undergoes Zn2+-dependent polymerization into filaments. KIR polymerization could represent a new transmembrane signaling mode, wherein a transmembrane receptor polymerizes into higher-order assemblies that are much larger than dimer or known oligomers of transmembrane receptors.

KIR signals through its cytosolic immunoreceptor Tyr-based inhibitory motifs (ITIM) (Figure 1). Upon recognizing HLA-C on target cells, KIR clusters at the resulting NK cell-target cell synapses (inhibitory synapses), and becomes ITIM-phosphorylated. The mechanisms underlying ITIM phosphorylation is unknown. Phosphorylated ITIMs (pITIMs) recruit and activate the protein Tyr phosphatase SHP-1. The molecular mechanisms that control recruitment and function of SHP-1 at inhibitory synapses are unclear. Activated SHP-1 dephosphorylates the guanine nucleotide exchange factor-1 (Vav-1). KIR engagement also involves c-Abl kinase-mediated phosphorylation of the adaptor protein Crk, and its dissociation from signaling complexes formed during activation. How c-Abl is recruited and activated at inhibitory synapses is unknown. Vav-1 dephosphorylation and Crk phosphorylation contribute to inhibition of actin-dependent activation signals.

We have three specific aims. (1) To understand the molecular events of KIR signaling. How ITIM phosphorylation is controlled and how the concerned kinase functions? How SHP-1 and c-Abl recruitment and function are controlled at inhibitory synapses? The tools of in vitro reconstitution, fluorescence imaging, and cellular biochemistry are being used to answer these questions. (2) To elucidate the mechanism of regulation and function of KIR polymers. We would use cellular biochemistry in conjunction with mass spectrometry to understand how KIR polymerization is controlled and how KIR polymers promote signaling. (3) It is highly likely that other transmembrane receptors also signal as polymers. KIR is the only known example so far. To obtain a consolidated understanding of this new mode of transmembrane signaling, we wish to obtain and study more examples.

T cell response and recognition in human *Helicobacter pylori* infection

H. pylori infection is the major cause of gastroduodenal pathologies that could lead to chronic gastritis and gastric cancer. T lymphocytes seem to play the central role in protection as well as progression of gastritis in *H. pylori* infections. A few antigenic proteins of *H. pylori*are seen to act as vaccines in mice. While specific T cell responses are triggered during *H. pylori* infections, peptide antigens of those T cells are not known. Many infected individuals (progressors) do not recover after antibiotic treatment and develop chronic gastritis. We wish to discover T cell antigens of *H. pylori* from active human infections, to test T cell-based autoimmune etiology of *H. pylori*-induced chronic gastritis, to discover peptide antigens of those self-reactive T cells (if any), and to understand what kinds of T cell responses determine whether or not an infected individual would return to health after antibiotic treatment.

We infected the human epithelial cell line AGS with *H. pylori*, isolated the HLA-bound peptides, and identified those peptides using mass spectrometry. We found peptides from three of the *H. pylori* proteins (urease, NAP, and aminopeptidase) that were presented by HLA on infected cells. We are working to make HLA tetramers with these peptides to detect the respective T cells in humans infected with *H. pylori*.

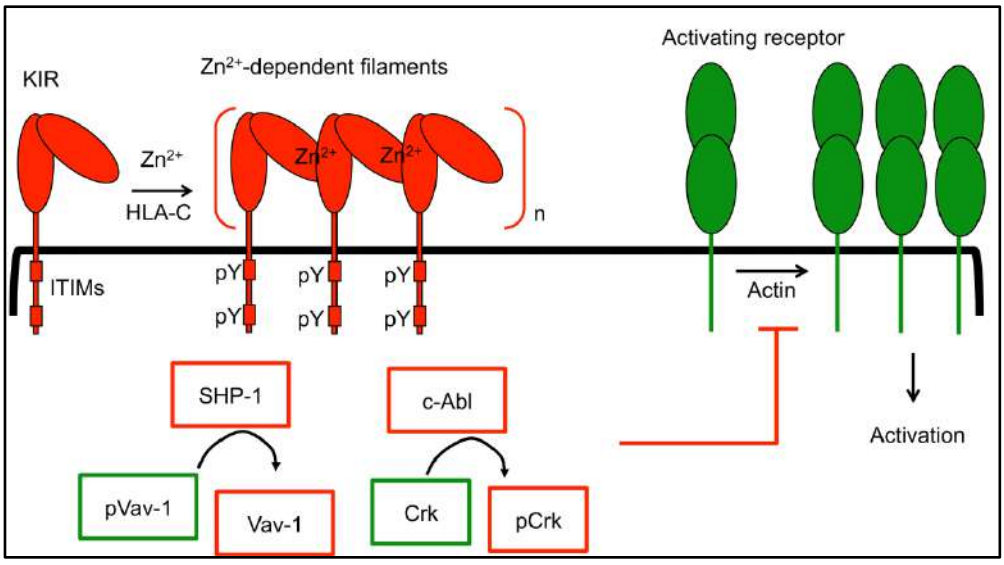


Fig. 1: Interception of NK cell activation by KIR. At the inhibitory synapses formed between KIR+ NK cells and HLA-C+ target cell, KIR clusters rapidly, in actin-independent manner. The Zn^{2+}-dependent polymerization of KIR into filaments could contribute to the rapid and actin-independent KIR clustering at these synapses. The Srk family kinase Lck and Fyn are candidate kinases for ITIM phosphorylation. The protein Tyr phosphates SHP-1, recruited and activated by its interaction with phospho-ITIMs, dephosphorylates the guanine nucleotide exchange factor Vav-1. The c-Abl kinase is recruited to the inhibitory synapses through an unknown mechanism. The c-Abl kinase phosphorylates the small adaptor protein Crk, and dissociates it from a signaling complex (not shown here) formed during activation. Vav-1 dephosphorylation and Crk phosphorylation contribute to blockage of actin-dependent signals for NK cell activation, and thus could contribute to inhibition of proximal actin-dependent steps, such as LFA-1 activation (not shown here) and clustering of activating receptors.

MUKESH LODHA

Mechanism of Epigenetic Inheritance in Plants

From left to right: Isha Joshi, Shraddha Lahoti, Preethi Jampala, Sharmila Singh, Aditya Undru, Mukesh Lodha, Sai Deep, Akansha Garhewal

RESEARCH INTERESTS:

- Mechanism of epigenetic inheritance
- Epigenetic regulations in root and leaf development
- Polycomb and Trithorax protein complexes

"We are interested in elucidating mechanisms of transmission of epigenetic memory from one cell to another, or one generation to the next. We use Arabidopsis thaliana as a model plant and use cell biology, molecular biology, biochemical and genetic approaches to investigate the mechanism of inheritance of epigenetic cellular memory"

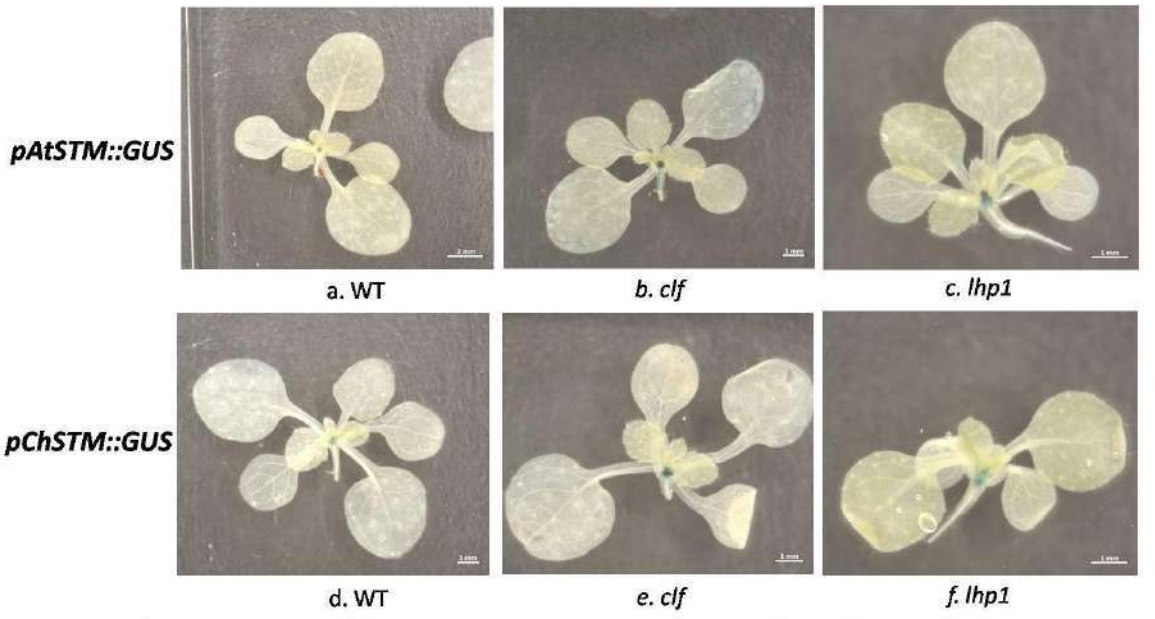
Selected recent publications

- Sarma S, Lodha M* (2017). Phylogenetic relationship and domain organisation of SET domain proteins of Archaeplastida. *BMC Plant Biology* 17: 238*Correspondence
- Lodha M, Marco CF, Timmermans MCP (2013). Direct Recruitment of Polycomb Repressive Complex2 by the *Arabidopsis* ASYMMETRIC LEAVES Complex. *Genes and Development* 27: 596-601.
- Husbands AY, Benkovics AH, Nogueira FT, Lodha M, Timmermans MC (2015). The ASYMMETRIC LEAVES Complex Employs Multiple Modes of Regulation to Affect Adaxial-Abaxial Patterning and Leaf Complexity. *Plant Cell* 27: 3321-3335.
- Lodha M*, Schulz-Raffelt M*, Schroda M (2008). A new assay for promoter analysis in *Chlamydomonas* reveals roles for heat shock elements and the TATA box in HSP70A promoter-mediated activation of transgene expression. *Eukaryotic Cell* 7: 172-176.* Equal contribution

- Lodha M, Schroda M (2005). Analysis of chromatin structure in the control regions of the chlamydomonas HSP70A and RBCS2 genes. *Plant Molecular Biology* 59: 501-513.

Epigenetic information is heritable during mitotic and/meiotic cell divisions but it is not encoded in the genetic material. It is stable even in the absence of an initial trigger and is reversible to various extents. Epigenetic regulation is important for heterochromatin maintenance and euchromatic gene regulation among many other cellular processes. We know fair bit about acquisition of epigenetic memory but an important question that remains underexplored is the mechanism of inheritance of the epigenetic state. Our group has a strong interest in elucidating mechanisms of transmission of epigenetic memory from one cell to another or one generation to the next. We use *Arabidopsis thaliana* as a model plant and take cell biology, molecular biology, biochemical and genetic approaches to address the question of mechanism of inheritance of epigenetic cellular memory. Histone post translational modifications, DNA methylation and non-coding RNA can potentially transmit memory during cell division. As a first step in our understanding the mechanism epigenetic inheritance, we are trying to identify histone tail residues which are important in epigenetic inheritance. Towards this goal, we have identified histone tail residues which are important in inheritance of epigenetic memory of cold and transmission of histones during cell division. Histone H3K23 and K14 are important in transmission of histone H3 during cell division and root development. Acetylation on these two residues appears to be important in histone H3 transmission during root development. We are also testing candidate protein complexes like trithorax and polycomb for their role in inheritance of epigenetic memory.

A large share of our understanding of epigenetics is achieved through developmental genes. Our group is using one of the important developmental regulator, SHOOT MERISTEMLESS (STM) as a tool to understand epigenetic regulation in plants. STM is an important shoot meristem stem cell regulator and a determining factors in leaf complexity. In simple leaves species such as *Arabidopsis thaliana,* STM is expressed in the shoot apical meristem and is downregulated in leaf primordia. This downregulation is maintained throughout the leaf development and is required for proper simple leaf development. In compound leaf species like *Cardamine hirsuta*, tomato and pea, *STM* downregulation occurs in leaf primordial but is not maintained. STM reactivation in developing leaves is necessary and sufficient for compound leaf formation. Remarkably the STM promoter from *Arabidopsis thaliana* (simple leaf species) can recapitulate the simple leaf STM expression pattern when introduced in *Cardamine hirsuta* (compound leaf species). Conversely, the STM promoter from *Cardamine hirsuta* can recapitulate compound leaf expression pattern when introduced in *Arabidopsis thaliana*. These experiments suggest that *cis* regulatory sequences of *Arabidopsis thaliana* and *Cardamine hirsuta* STM promoters are important in determining their expression patterns and thereby leaf complexity. There are indications that STM is downregulated in the leaf primordia in simple leaf species like *Arabidopsis thaliana* by transcriptional repressors such as the Polycomb complexes. It is tempting to hypothesize that cis regulatory elements binding to PRC2 / transcriptional repressors restricts STM expression only to the shoot apical meristem in simple leaf species and such *cis* regulatory sequences are absent in compound leaf species. We have partly identified and characterised the *cis* regulatory elements in the simple leaf species *Arabidopsis thaliana* and compound leaf species *Cardamine hirsuta* which allow differential expression of STM in these two species, as model of leaf complexity in plants and to establish the role of chromatin in differential regulation of STM (in figure).



pAtSTM :: GUS and *pChSTM :: GUS* promoter reporter (Promoters of SHOOT MERISTEMLESS of *Arabidopsis thaliana* and *Cardamine hirsuta* driving GUS reporter) transgenics in Col WT (a and d); *clf* (b and e); *lhp1* (c and f) respectively. *A. thaliana* (simple leaf) promoter elements are more responsive to Polycomb Repressive Complex 2 (PRC2) group proteins than *C. hirsuta* (compound leaf) promoter elements (Compare b and e; c and f). Ectopic expression pattern of *A. thaliana* reporter in Polycomb mutant background is similar to the endogenous expression pattern in compound leaf species (compare b & c to d) implying that Polycomb group proteins play a role in leaf complexity determination

M M IDRIS

Bio-mechanisms of Regeneration

M M Idris

RESEARCH INTERESTS:

- Understanding the biomechanism of tissue regeneration in alternate model animals.
- Understanding the molecular perspective duringwound healing and regeneration in zebrafish model system.
- Development of Primary reference standard and impurities for biopharmaceuticals.
- Development of DNA barcode and Monograph for herbal drugs.

"Our group works on understanding the biomechanism of epimorphic regeneration of appendages in vertebrate and invertebrate model animals. We are also involved in development of primary reference standards, impurities, monograph for therapeutic proteins and herbal drugs."

Selected recent publications

- Rekulapally R, Murthy Chavali LN, Idris MM, Singh S (2019). Toxicity of TiO2, SiO2, ZnO, CuO, Au and Ag engineered nanoparticles on hatching and early nauplii of Artemia sp. *PeerJ* 6: e6138.

- Quoseena M, Vuppaladadium S, Hussain S, Bharathi S, Idris MM (2018). Functional role of Annexins in Zebrafish Caudal fin regeneration: A Gene knockdown approach in regenerating tissue. *BioRixV* 342228.

- Babu NS, Murthy CL, Kakara S, Sharma R, Swamy CV, Idris MM (2016). MPTP induced Parkinson's disease in zebrafish. *Proteomics* 16: 1407–1420.

- Saxena S, Purushothaman S, Meghah V, Bhatti B, Poruri A, Meena Lakshmi MG, Babu NS, Murthy CL, Mandal KK, Kumar A, Idris MM (2016). Role of Annexin gene and its regulation during zebrafish caudal fin regeneration. *Wound, Repair and Regeneration* 24: 551-559.

- Purushothaman S, Saxena S, Meghah V, Swamy CVB, Ortega-Martinez O, Dupont S, Idris MM (2015). Transcriptomic and proteomic analyses of Amphiurafiliformis arm tissue-undergoing regeneration. *Journal of Proteomics* 112: 113-124.

Bio-mechanisms of Regeneration

Our group works on understanding the complexity of developmental biology and neuroscience involved in the biomechanism of regeneration and degeneration processes. We are trying to unravel the molecular and genetic aspects involved in tissue regeneration and degeneration among alternate model animals like zebrafish, ascidians and echinoderms. Understanding the bio-mechanisms of regeneration and the association of various genes (or proteins) in the regenerating environment is of high significance, as it might help us engineer non-regenerating systems into regenerating systems for therapy and healing. Understanding the regeneration mechanisms among different model animals might lead to an overall better understanding of the underlying mechanisms.

We are focusing on the regeneration of appendages in zebrafish, nervous tissues in ascidians and arms in starfish using proteomics and transcriptomics approaches. We have identified several proteins including Annexin 1, 2, and 5 as differentially regulated or posttranslationally modified during zebrafish caudal fin regeneration. The proteins were differentially expressed in the regenerating tissues either immediately after amputation (1-hour post amputation (hpa)) or later during regeneration (12, 24, 48 and 72 hpa). Similarly, we have identified several novel transcription factors and epigenetic modifiers undergoing differential regulation during regeneration. Role of epigenetic regulatory mechanisms, such as histone H3 and H4 lysine acetylation and methylation during zebrafish caudal fin regeneration were also studied involving ChIP and RTPCR assay. More than 700 genes were identified as differentially regulated in the echinoderm arm regeneration based on high throughput transcriptome mapping and nearly 200 proteins were identified as differentially expressed in the nervous system of ascidians during regeneration.

During zebrafish caudal fin regeneration several gene families such as PRMT, HOX, Interleukins and Neurotransmitters were found differentially regulated. These genes were found differentially regulated for different regeneration time points and wound healing. Our study also identified the functional role of Annexin genes (Annexin 2a and 2b gene) in regeneration through a novel CRSISPR-Cas9 based gene knock down approach in the adult zebrafish fin tissue. We found retarded growth in caudal fin tissue when *Annexin 2a* and *2b* genes were knocked down (Figure 1), which was validated through gene expression analysis and further supported by high-throughput quantitative proteomic analysis of fin tissue. Annexin family genes such as ANXA13, ANXA1a, ANXA5b were also found to be repressed in their expression. Knocking down of *ANXA2a* and *2b*in regenerating caudal fin tissue leads to compromised regenerating capacity as these genes were found to be involved in cell to cell communication and extracellular matrix growth. This study proves that ANXA2a and 2b plays a significant role in epimorphic regeneration of zebrafish caudal fin tissue.

Biologics

Our group also works on the development of primary reference standards for biopharmaceuticals, monograph development of monoclonal antibodies and DNA barcode development for medicinal plants as per the requirement of Indian Pharmacopeia commission. This initiative for the developmentof reference standards and Monographs for biotherapeutics might strengthen our country's program on affordable health and helps to produce quality biotherapeutics drugs under Ayushman Bharath.

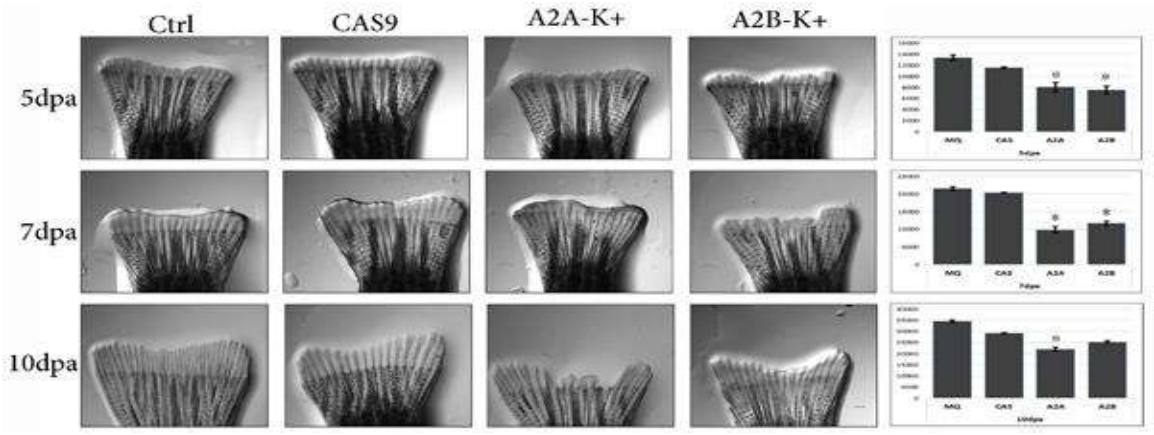


Fig. 1: Phenotype of regenerating caudal fin tissue at 5, 7 and 10 dpa electroporated with water, Cas9, ANXA2a and ANXA2b CRISPR targets. B. Bar diagram detailing the rate of fin tissue growth at respective time points and electroporation.

M V JAGANNADHAM

Studies on outer membrane vesicles of Bacteria

From left to right: M.V. Jagannadham, Meghana, Deepika Chandra, Shabnam Chandel

RESEARCH INTERESTS:

- Structural and functional studies on outer membrane vesicles of bacteria
- Proteomics of an Antarctic bacterium *Pseudomonas syringae* Lz4W
- Improving *de novo* sequencing efficiency of peptides using MS

"Twenty two proteins detected earlier in human transcriptome studies were for the first time identified from tumour cell lines Hela, MCF 7 and BT474 at the protein level using LC-MS/MS providing the analytical basis for their expression."

Selected recent publications

- Karthikeyan R, Gayathri P, Gunasekaran P, Jagannadham MV, Rajendhran J (2019). Comprehensive proteomic analysis and pathogenic role of membrane vesicles of *Listeria monocytogenes* serotype 4b reveals proteins associated with virulence and their possible interaction with host. *International Journal of Medical Microbiology* 309: 199-212.
- Chandra D, Gayathri P, Vats M, Nagaraj R, Ray MK, Jagannadham MV (2019). Mass spectral analysis of acetylated peptides: Implications in proteomics. *European Journal of Mass spectrometry* (In press).
- Bina A, Karthikeyan R, Gayathri P, Rameshbabu B, Ahmed G, Jagannadham MV (2019). Studies on the mechanism of multidrug resistance of *Acinetobacter baumannii* by proteomic analysis of the outer membrane vesicles of the bacterium. *Journal of Proteins and Proteomics* 10: 1-15.
- Doddi SK, Kummari G, Jagannadam MV, Kalle AM (2019). Protein kinase A-mediated novel Serine 584

phosphorylation of HDAC4. *Biochemistry and Cell Biology* (In press)

- Neelam D, Agrawal A, Tomer A, Jagannadham MV, Bandyopadhayaya S, Sharma A, Mandal C, Dadheech P (2019). Characterization, Phylogenetic Analysis of Newly Isolated *Piscibacillus* sp. Strain C12A1 (a Moderately Halophilic Bacterium) from a Soda Lake and its Anticancer Activity Against Breast Cancer MDA-MB-231 cells. *Microorganism 7*: 34.

In mass spectrometry based proteomics, sequence determination of peptides using mass spectrometry plays a critical role in the bottom-up approaches for the identification of proteins. It is important to minimise false detection and validate the sequence of the peptides for correct identification of a protein. Chemical modification of peptides followed by mass spectrometry is an option for improving spectral quality. *In silico*derived tryptic peptides with different N-terminal amino acids were designed from human proteins and synthesized. The effect of acetylation on the fragmentation of peptides was studied. N-terminal acetylation of the tryptic peptides was shown to form b1-ions, and improve the abundance and occurrence of b-ions. In some cases, the intensity and occurrence of some y-ions also varied demonstrating that acetylation plays an important role inimproving the *de novo* sequencing efficiency of the peptides.

The acetylation method was extended to tryptic peptides generated from the proteome of an Antarctic bacterium *Pseudomonas syringae* Lz4W grown at 4^0C using the proteomics work flow and mass spectra of the peptides were analysed. Comparison of the MS/MS spectra of the acetylated and unacetylated peptides revealed that acetylation helped in improving the spectral quality and validated the peptide sequences. Using this approach 673 proteins of the 1070 proteins identified were validated. Acetylation is a simple reaction and can be applied to proteome level as is shown in the present study. Validation of peptide sequences using this method helps in the accurate establishment of the proteome.

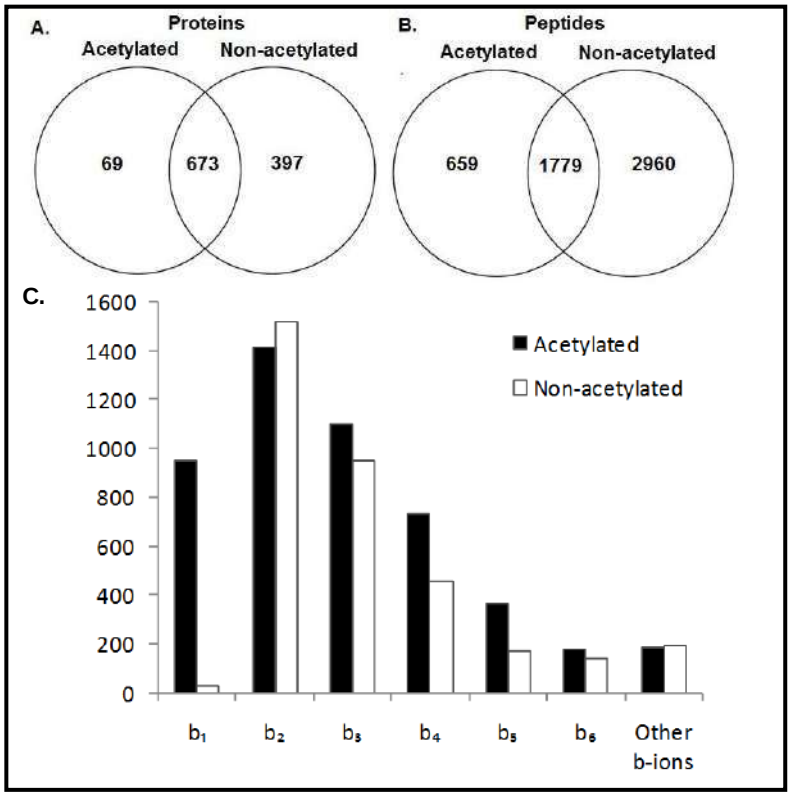


Fig. 1: The numbers of proteins validated are shown in figure A. The number of common peptides identified between normal and acetylated peptides are shown in panel B. Panel C depicts the variation in the intensity of the number of tryptic peptides.

Identification of human hypothetical proteins

A myriad of predicted proteins have been described at the genome scale but their existence has not been confirmed at the protein level. Proteins that are predicted to be expressed from an open reading frame (ORF) but for which translation has not been demonstrated are known as hypothetical proteins and constitute a major fraction of the human proteome. In this study we identified and characterized hypothetical proteins from human tumor cell lines, viz., HeLa, MCF7 and BT474, thus providing the analytical basis for their expression. We used gel electrophoresis followed by *in gel* digestion of the protein lanes and subsequent LC-MS/MS analysis of protein tryptic digests.On searching against human hypothetical protein data from NCBI database, 110 common proteins were identified across the three selected cells lines. 22uncharacterized protein sequences that have not been described before at the protein level were identified for the first time.These were subjected to functional annotation using bioinformatics methods and available databases. This allowed us to build functional hypotheses for twelve uncharacterized human proteins. These hypotheses cover the functions of cell adhesion, DNA repair, mRNA splicing, RNA binding, transcription, membrane transport, ubiquitin binding and cell signalling. We conclude that the proteomic approach used would serve as a suitable tool to validate the existence of predicted or hypothetical proteins at the protein level thus providing a clue for their functional role. The MS proteomics data have been deposited in the ProteomeX change consortium via PRIDE with the data set identifiers PXD014258.

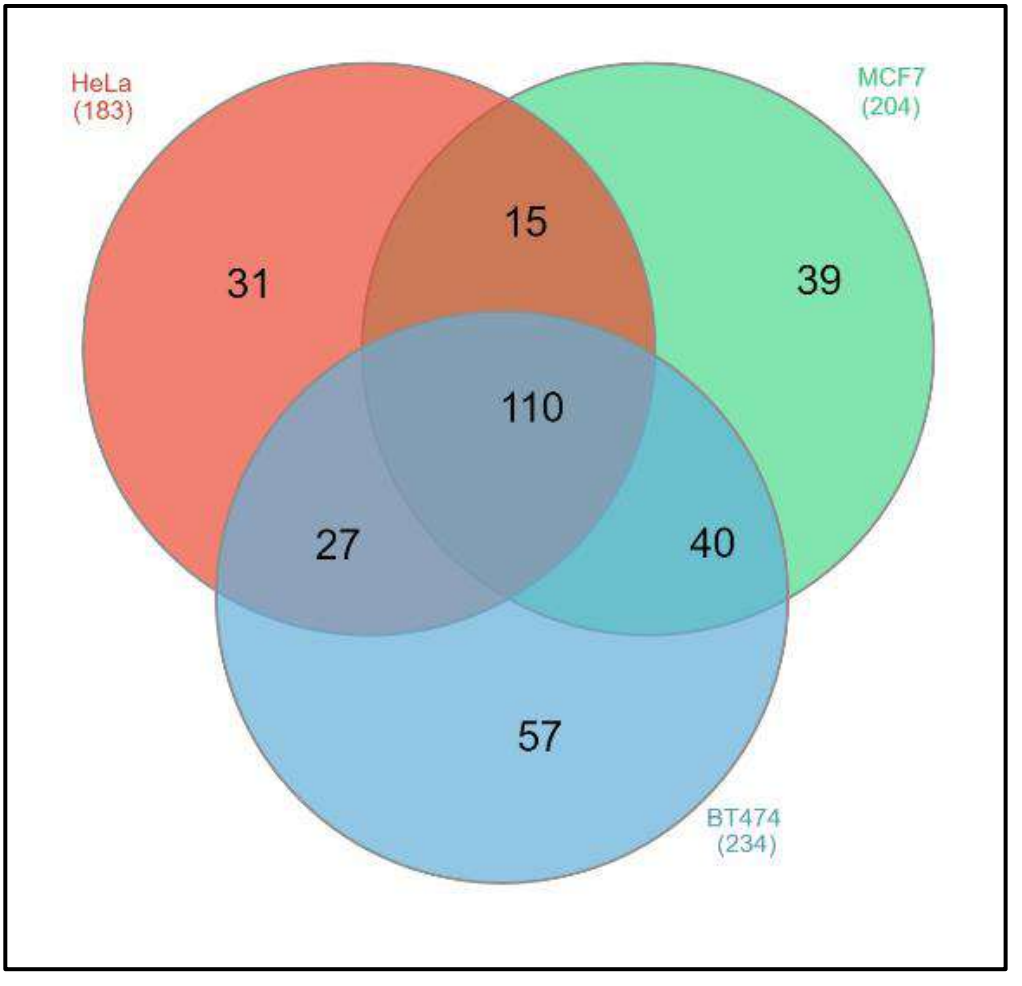


Fig. 2: Common hypothetical proteins identified from the three cell lines.

RAKESH K MISHRA

Genome Organization and Epigenetic Regulation

From left to right: Ravina Saini, Akshay Avvaru, M. Nisha, Sofia Banu, Sangam, M. Soujanya, Divya Tej Sowpati, Runa Hamid, Ashish Bihani, Parna Saha, Shagufta Khan, Avinash Srivastava, Rakesh Mishra, Shreekant Verma, A. Srinivasan, R. Phanindhar, Rashmi U. Pathak, Sonu Yadav, Nikhil Hajirinis

RESEARCH INTERESTS:

- Comparative and functional genomics of non-coding DNA
- Organization & regulation of *Hox*genes: evolutionary logic of animals body plan
- Epigenetic regulation and development.

"We have developed a comprehensive database of Microsatellites by analysing their patterns of distribution across eukaryotic genomes and uncovered novel functional and evolutionary aspects of this part of the genome. Another highlight is our analyses of heterochromatic regions of the genome that has led us to propose diverse roles for non-coding transcripts from constitutive heterochromatin. We have also recently reviewed various aspects of mechanisms of regulation of homeotic genes in the perspective of their genome-wide implications in complex regulatory processes"

Selected recent publications

- Akshay Kumar Avvaru, Deepak Sharma, Archana Verma, Rakesh K. Mishra, Divya Tej Sowpati (2019). MSDB: a comprehensive, annotated database of microsatellites. *Nucleic Acids Research DOI: 10.1093/nar/gkz886*
- Indira Paddibhatla, Dushyant K. Gautam, Rakesh K. Mishra (2019). SETDB1 modulates the differentiation of both the crystal cells and the lamellocytes in Drosophila. *Developmental Biology* 456: 74-85.
- Surabhi Srivastava, Akshay Kumar Avvaru, Divya Tej Sowpati, Rakesh K. Mishra (2019). Patterns of microsatellite distribution across eukaryotic genomes. *BMC Genomics* 20: 153.
- Runa Hamid, Nikhil Hajirnis, Shikha Kushwaha, Sadaf Saleem, Vimlesh Kumar, Rakesh K. Mishra (2019). Drosophila Choline transporter non-canonically regulates pupal eclosion and NMJ integrity through a neuronal subset of mushroom body. *Developmental Biology* 446: 80-93.

- Parna Saha, Rakesh K Mishra (2019). Heterochromatic hues of transcription- the diverse roles of non-coding transcripts from constitutive heterochromatin. *FEBS Journal* DOI:10.1111/febs.15104

Packaging of genomic DNA has regulatory consequences on the expression of genes during development. This regulation is based on chromatin structure in which organization of coding and non-coding elements of the genome plays an important role. A good example of such regulation is provided by the Hox cluster that shows a colinearity of gene expression pattern with the arrangement of the genes in the cluster, a feature known to be conserved in all bilaterians. Chromatin domain boundary elements, the topologically independent structural unit of higher order chromatin organization, and cellular memory elements, that maintain the expression state of genes by means of chromatin structure, regulate the expression of homeotic genes. Such epigenetic regulatory mechanisms control genes at many loci in the eukaryotic genome and have been found to be conserved during evolution. Our group is interested in understanding how genetic information in the form of genomic sequence is interpreted by developmental mechanisms and how cell type-specific packaging of the genome in the context of nuclear architecture is achieved and maintained throughout the life of the individual. Some of our findings during the period of this report are described below.

Microsatellites: a comprehensive database and patterns of distribution across eukaryotic genomes

Microsatellites, or Simple Sequence Repeats (SSRs), are short tandem repeats of 1-6 nt motifs present in all genomes. Emerging evidence points to their role in cellular processes and gene regulation. Despite the huge resource of genomic information currently available, SSRs have been studied in a limited context and compared across relatively few species. We have identified ~685 million eukaryotic microsatellites and analyzed their genomic trends across 15 taxonomic subgroups from protists to mammals. The distribution of SSRs reveals taxon-specific variations in their exonic, intronic and intergenic densities. Our analysis reveals the differences among non-related species and novel patterns uniquely demarcating closely related species. We document several repeats common across subgroups as well as rare SSRs that are excluded almost throughout evolution. We further identified species-specific signatures in pathogens like *Leishmania* as well as in cereal crops, *Drosophila*, birds and primates. We also find that distinct SSRs preferentially exist as long repeating units in different subgroups; most unicellular organisms show no length preference for any SSR class, while many SSR motifs accumulate as long repeats in complex organisms, especially in mammals. We carried out a comprehensive analysis of SSRs across taxa at an unprecedented scale. Our analysis indicates that the SSR composition of organisms with heterogeneous cell types is highly constrained, while simpler organisms such as protists, green algae and fungi show greater diversity in motif abundance, density and GC content. The microsatellite dataset generated in this work provides a large number of candidates for functional analysis and for studying their roles across the evolutionary landscape. We have also made a 'MicroSatellite DataBase'(MSDB) with a collection of >4 billion microsatellites from 37,680 genomes presented in a user-friendly web portal for easy, interactive analysis and visualization.This is by far the most comprehensive, annotated, updated database to access and analyse microsatellite data of multiple species. The features of MSDB enable users to explore the data as tables that can be filtered and exported, and also as interactive charts to view and compare the data of multiple species simultaneously. Its modularity and architecture permit seamless updates with new data, making it a powerful tool and useful resource to researchers working on this important class of DNA elements, particularly in context of their evolution and emerging roles in genome organization and gene regulation (Fig 1).

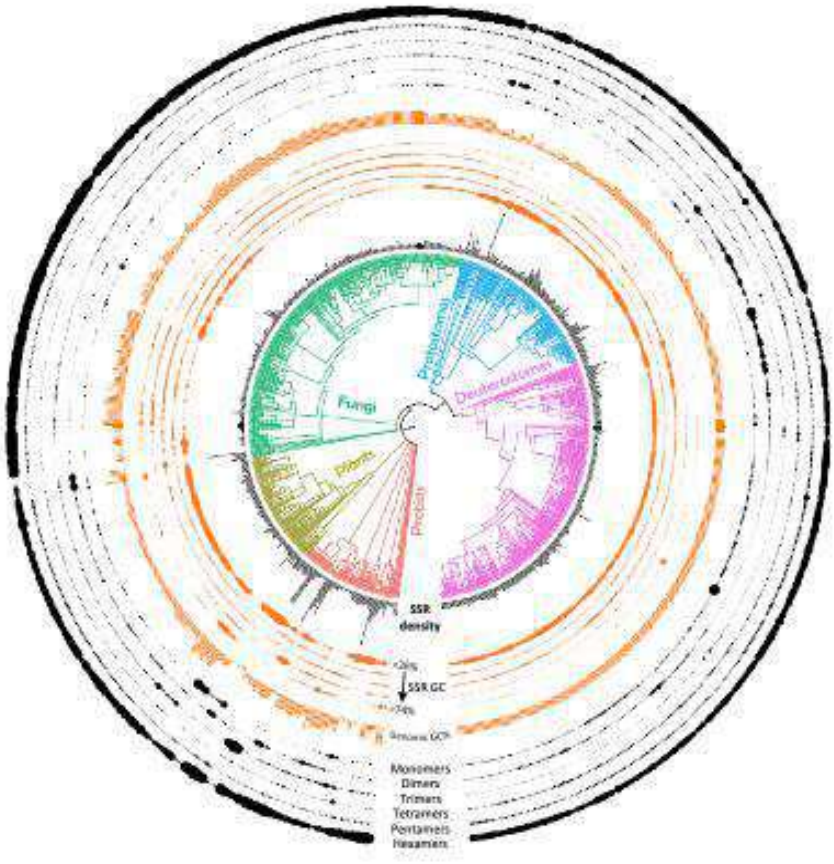


Fig. 1: Phylogenetic tree representation summarizing attributes of all SSRs analysed. The tree was constructed using iTOL (interactive Tree Of Life) webserver. The clade nodes are coloured based on the 5 groups used in this study. Black bars (the innermost track) around the organisms represent the SSR density (bases covered per MB of genome) in each organism. The orange tracks around the SSR density show the SSR GC% in each organism (the innermost orange track represents the relative enrichment of motifs with <=25% GC, while the outermost orange track represents SSR GC >=75%) and the middle three tracks represent intermediate GC ranges. The size of each dot on the track (representing each organism) indicates the amount of SSRs present in that GC range. The orange bars represent the genomic GC content. The black tracks show the distribution in each organism based on the motif size of the repeat (the innermost black track represents monomers while the outermost black track represents hexamers). The size of each dot on the track (representing each organism) indicates the proportion of SSRs present in that motif size range.

Heterochromatic hues of transcription- the diverse roles of non-coding transcripts from constitutive heterochromatin

Constitutive heterochromatin has been canonically considered as a transcriptionally inert chromosomal region, which silences repeats and transposable elements to preserve genomic integrity. However, several studies from the last few decades show that centromeric and pericentromeric regions also get transcribed and these transcripts are involved in multiple cellular processes. The regulation of such spatially and temporally controlled transcription and their relevance to heterochromatin function have emerged as an active area of research in chromatin biology. We have reviewed the myriad roles of non-coding transcripts from the constitutive heterochromatin in the establishment and maintenance of heterochromatin, kinetochore assembly, germline epigenome maintenance, early development, and diseases. Contrary to general expectations, there are active protein-coding genes in the heterochromatin- although the regulatory mechanisms of their expression are largely unknown. We propose plausible hypotheses to explain heterochromatic gene expression using *Drosophila melanogaster* as a model and discuss the evolutionary significance of these transcripts in the context of Drosophilid speciation. Such analyses offer insights into the regulatory pathways and

functions of heterochromatic transcripts that open up new avenues for further investigation (Fig. 2).

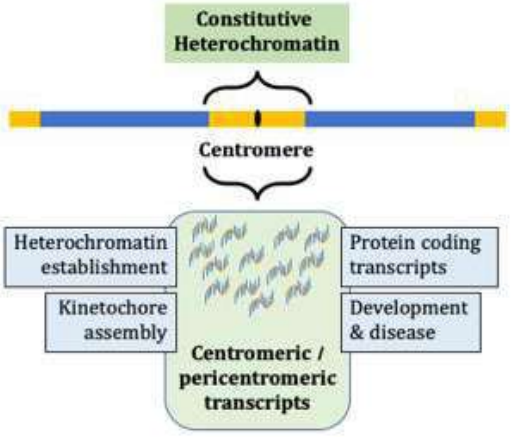


Fig. 2: Heterochromatin and the ncRNA. Constitutive heterochromatin produces ncRNA in a highly regulated manner that are involved in cellular processes like heterochromatin establishment, kinetochore assembly and maintenance of the epigenome status in soma and germline. Heterochromatic transcripts have also been implicated in early development and various diseases. The generally repressive constitutive heterochromatin, also harbors active genes and mechanistic insight in their regulation is emerging from the studied using *Drosophila* as the model system.

Lessons on gene regulation from the bithorax complex

Conservation of genes and also that of their arrangement suggests a linkage between co-regulation and the higher order chromatin organization of the homeotic gene complex in bilaterians. To this end, we and others have used *Drosophila* as the model system to understand the cis-and trans-regulatory components of Hox genes. A number of chromatin-level regulatory elements, like chromatin domain boundaries, and Polycomb Response Elements (PREs) have been discovered in this process. Interestingly, much of what has emerged from the study of homeotic genes, the cis-elements and protein factors, have relevance across the genome in a large number of regulatory events beyond the Hox genes. We have recently reviewed our findings and discuss their genome-wide implications in complex regulatory processes. [Srinivasan & Mishra (2019), The International Journal of Developmental Biology, in press]. We propose that the new findings will help us solve the mystery of the mechanism that involves collinearity of *Hox* gene organization and function which turn out to be the only way for bilaterians to achieve A-P body axis formation. Understanding of such complex regulatory processes provides insight into the evolution of developmental mechanisms that contributed to the evolution of variety and complexity in living systems (Fig. 3).

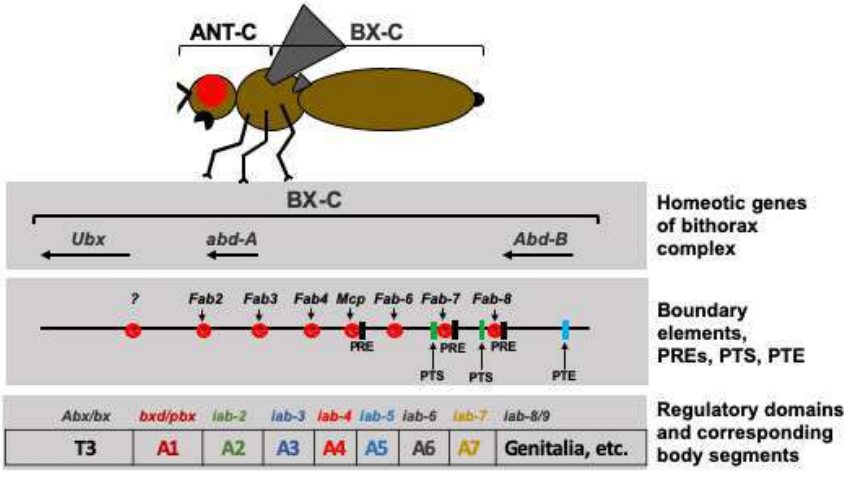


Fig. 3: Homeotic genes and regulation of A-P body axis. In *Drosophila melanogaster*, the Hox genes are clustered in two complexes, antennapedia complex (ANT-C) and bithorax complex (BX-C), which determine the identity to the body segments in anterior and posterior parts, respectively. BX-C determines the segmental identity from the third thoracic to the posterior end of the body. The three homeotic genes, *Ubx*, *abd-A* and *Abd-B*, exist in an order collinear to their function and so are the nine cis-regulatory domains that determine the identity of corresponding body segments. Cis-regulatory domains are defined by the boundary elements. The representative boundaries, PREs, PTS, PTE are shown as red circle-backslash symbols, black, green and blue vertical lines respectively (not to scale).

P CHANDRA SHEKAR

Early Embryonic Development in Mouse

From Left to Right: Preeti Mourya, Anusha Domreddy, Debabrata Jana, Hanuman Kale (Front Row) Vijayram Ganasala, Vishnu Vijay, Nithya Priya, Mansi Srivastava, P. Chandra Shekar, Rajendra Singh (Back Row)

RESEARCH INTERESTS:

- Understanding the regulation of the core pluripotency factor Nanog in different pluripotent states.
- Identifying the barriers in ES cells for transition from pluripotent state to totipotent state.
- Studies on the process of self-organisation of preimplantation embryo like structures from pluripotent and totipotent cells.

"We are trying to understand cell fate choice based on transcription factor modulation in the pluripotent state. We are also studying the molecular mechanisms of pluripotency state transition and self-organisation of cell types from preimplantation embryonic stages to embryo like structures."

Selected recent publications

- Miguel Fidalgo*, P. Chandra Shekar*, Yen-Sin Ang, Yuko Fujiwara, Stuart H. Orkin, Jianlong Wang; (2011). Zfp281 Functions as a Transcriptional Repressor for Pluripotency of Mouse Embryonic Stem Cells. *Stem Cells* 29: 1705–1716. * Equal Contribution
- P Chandra Shekar, Adnan Naim, D Partha Sarathi, Satish Kumar; (2011). Argonaute-2-null embryonic stem cells are retarded in self-renewal and differentiation. *Journal of Bioscience* 36: 649-657.
- P. Chandra Shekar, Sandeep Goel, S. Deepa Selvi Rani, D. Partha Sarathi, Jomini Liza Alex, Shashi Singh, Satish Kumar (2006). κ-Casein-deficient mice fail to lactate. *Proc. Natl. Acad. Sci. USA* 103: 8000-8005.

Our group investigates how cell fate decisions are made in the early stages of embryogenesis and during stem cell differentiation based on the expression levels of key transcription factors. We use functional analysis and biochemical approaches to perturb transcription factor network to understand underlying mechanisms. We are studying an extended regulatory network comprising signalling pathways and transcription factors that maintain the expression levels of core pluripotency factors in self-renewal limits. We are using CRISPR based approaches to achieve site directed point mutations, Knock-in and deletions in the endogenous locus to engineer embryonic stem cells. These ES cell lines can report the activity of promoters, protein stability and dynamics of the core pluripotency factors like OCT4 and NANOG.

Regulation of the core pluripotency factor Nanog by phosphorylation

We have earlier reported that Nanog binding on the Oct4 promoter does not change with concentration, however Oct4 binding to the nanog promoter and its activation are dependent on the concentration of the Oct4 protein. The core transcription factors are not only regulated by transcription but their protein activity/levels in the cells are actively maintained by posttranslational modification. We have identified novel tyrosine phosphorylation of Nanog, which affect the stability of Nanog as well as cell fate choice of ES cells. The phosphomimetic of Nanog at a critical tyrosine residue destabilizes Nanog and leads to its degradation and differentiation of ES cells to primitive endoderm. We have identified the Tyrosine kinase that phosphorylates a Tyrosine residue and destabilises Nanog protein. This Tyrosine kinase is sensitive to the mechanical signals and hence determines the spatial distribution of Nanog protein among different cells in the colony or in a differentiating embryoid body.

Nanog auto repression

The Nanog mediated auto repression loop ensures that the expression level of Nanog does not exceed the normal self-renewal limits. We have identified a downstream target of Nanog which gets transcriptionally activated in just 1 hour of induction of Nanog. Further our experiments suggest that the downstream target is essential for auto repression of Nanog. This suggests that Nanog does not repress itself but it does so through a downstream target gene. Our experiments have identified a cascade of events, which lead to repression of Nanog at transcriptional and post translational levels. Our results also suggest that Nanog acts as only a trigger for the nanog autorepression loop but is not required for the execution of the repression. Further we have discovered that the Nanog autorepression is a cell non-autonomous process involving autocrine signalling. We are currently studying the downstream proteins of the autocrine signalling involved in Nanog autorepression.

Mechanisms regulating state transition of pluripotent and extended pluripotentstem cells

ES cells can differentiate to all the 3 germ layersof the body -ectoderm, mesoderm and endoderm. However ES cells cannot give rise to all extra embryonic layers particularly the trophoblast. Hence they are not totipotent like a Zygote. We had developed a new culture condition where a small population of ES cells differentiates to a trophoblast lineage. The ES cells not only differentiated to a trophoblast linage *in vitro* but they also contributed to the trophoblast of a developing blastocyst when the treated ES cells were injected into the morula. Further by genome editing tools we have identified the pathways through which a small molecule operates to induce trophoblast potential in ES cells. This molecule intervenes in these repression pathways and potentiate the ES cells to differentiate to trophoblast lineage. Based on this novel culture condition we have been able to develop methods to self-organise the pluripotent stem cells into blastocyst like structures. These blastocyst like structures show expression typical markers like Oct4 in inner cells and Cdx2 in the outer cells. Interestingly the blastocyst like structures could implant into the uterus of the pseudo pregnant mice and form deciduae and abort after 7.5 dpc (Fig 1). Currently we are improving the culture conditions for self-organisation to prolong the development of the implanted blastocyst like structures.

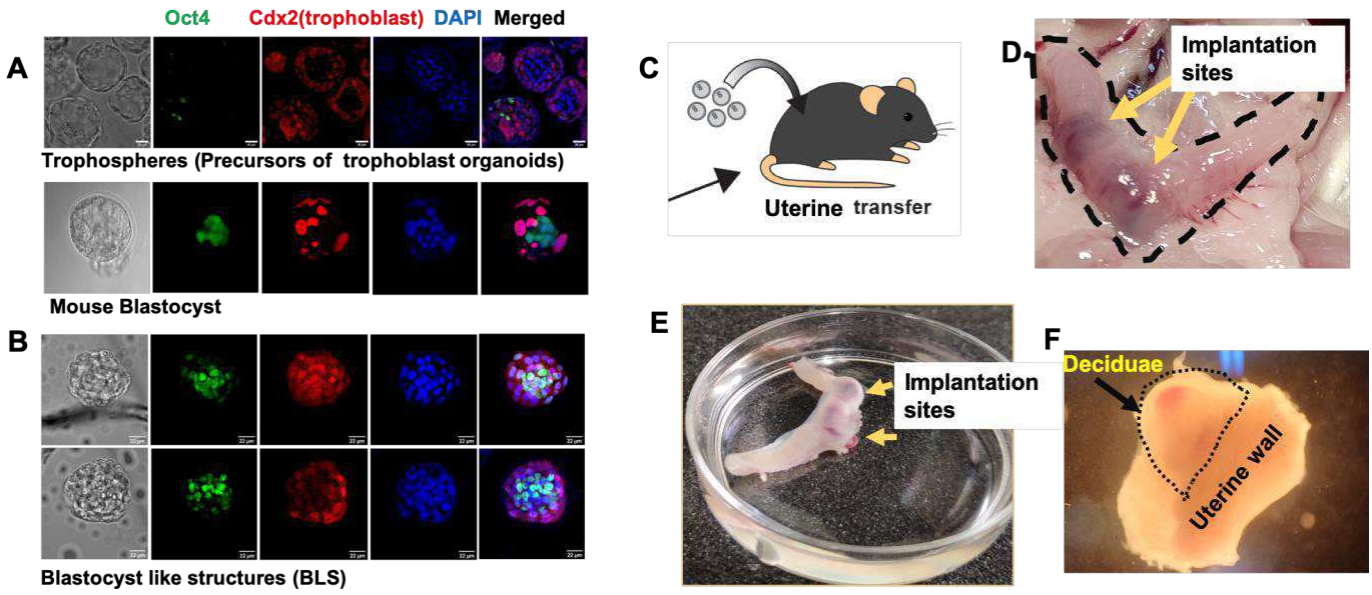


Fig. 1: Self organization of pluripotent stem cells into blastocyst like structures and troposphere. (A)Trophospheres with fluid filled cavity derived from pluripotent stem cells, (B) blastocyst like structures formed by self organization of pluripotency stem cells . The blastocyst like structures resemble mouse blastocyst with outer Cdx2 positive trophoblast and inner cell mass with Oct4. (C) Transplantation of the blastocyst like structures into uterus of pseudo pregnant mice. (D) site of implantation of Blastocyst like structures (E, F) Uterine wall showing decidualization.

VEENA K PARNAIK

Nuclear Organization and Lamin Biology

From left to right: Richa Khanna, Veena K Parnaik, Vidhya Krishnamoorthy, Ajay D Verma

RESEARCH INTERESTS:

- Studies on Ubiquitin-Proteasome signalling pathways in nuclear organization in laminopathies

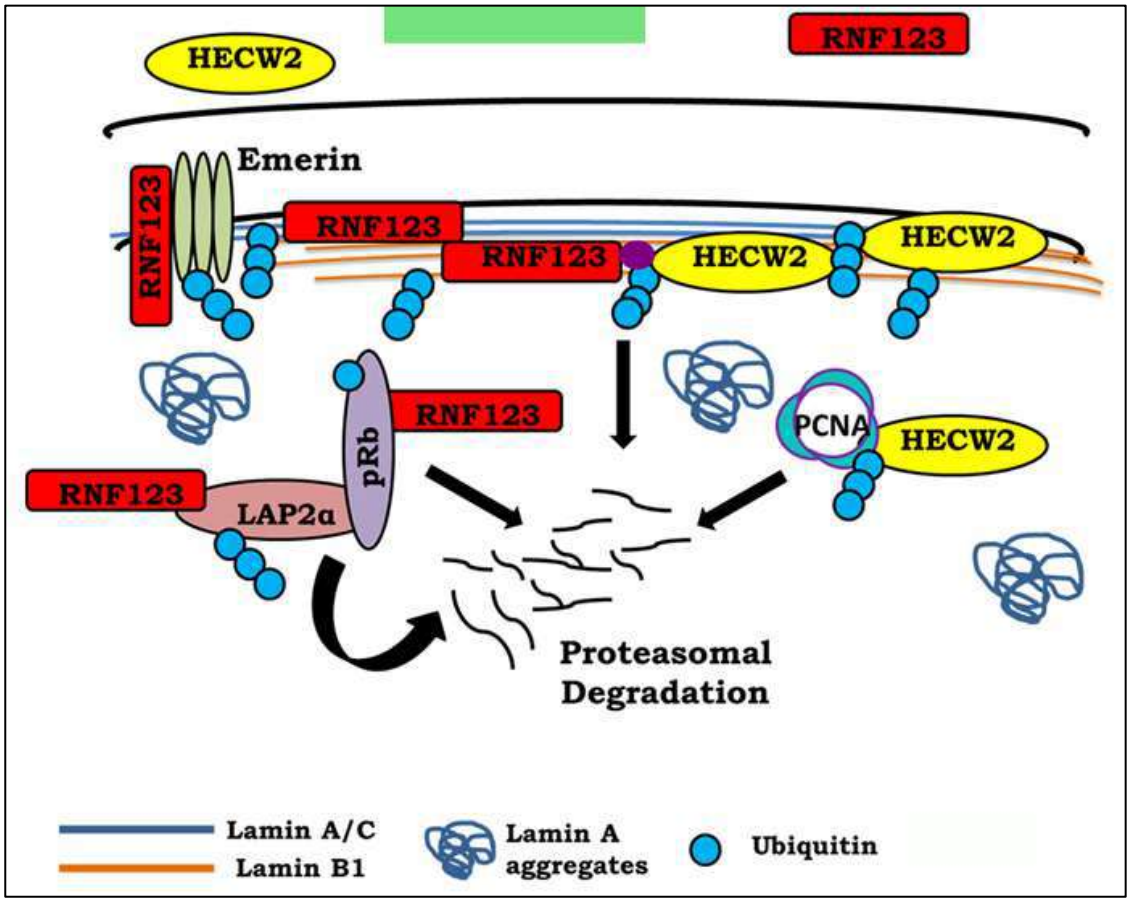
"We are interested in deciphering the role of E3 ubiquitin ligases in the pathology of diseases caused by mutations in nuclear lamina protein, Lamin A/C"

Selected recent publications

- Khanna R, Krishnamoorthy V, Parnaik VK (2018). E3 ubiquitin ligase RNF123 targets lamin B1 and lamin-binding proteins. *FEBS Journal* 285: 2243-2262.
- Krishnamoorthy V, Khanna R, Parnaik VK (2018). E3 ubiquitin ligase HECW2 targets PCNA and lamin B1. *BBA - Molecular Cell Research* 1865: 1088-1104.
- Krishnamoorthy V, Khanna R, Parnaik VK (2018). E3 ubiquitin ligase HECW2 mediates the proteasomal degradation ofHP1 isoforms. *Biochemical and Biophysical Research Communications* 503 (4): 2478-2484.

The nuclear lamina is a filamentous structure present underneath the inner nuclear membrane in metazoan cells and is primarily composed of lamin A/C and other lamin-binding proteins.The nuclear lamina plays a crucial role in maintaining nuclear structure and other crucial cellular processes,such as DNA replication, chromatin dynamics, and DNA damage repair.Therefore, mutations in lamins and other lamin-binding proteins cause a group of debilitating genetic diseases cumulatively termed as laminopathies.

The cellular hallmarks of laminopathies include dysmorphic nuclei, decreased heterochromatin, defective cell cycle, and increased proteasomal turnover of nuclear proteins. We recently identified that two E3 ligases, HECT domain ligase, HECW2 and RING domain ligase RNF123, are upregulated in cells expressing Emery-Dreifuss muscular dystrophy (EMD) causing rod domain mutations in Lamin A/C.We further showed that both, HECW2 and RNF123 mediate the ubiquitination and proteasomal degradation of key nuclear proteins, such as nuclear lamina protein lamin B1, cell cycle regulatory protein retinoblastoma, DNA replication processivity factor proliferating cell nuclear antigen (PCNA), and other lamin-binding proteins. Moreover, increased degradation of the substrates of these ligases predisposed cells to increased DNA damage, and altered cell cycle kinetics. The increased turnover of these proteins has been also been observed in laminopathies, and we speculate that increased UPS signaling might contribute to these phenotypes in laminopathic cells.



ANANT B PATEL

Brain Energy Metabolism in Neurological and Psychiatric Disorders

From left to right: Varadarajan, Dipak, Shibani, Bedaballi, Akila, Anant, Narayan, Bhargirdhar and Kamal

RESEARCH INTERESTS:

- Brain Energy Metabolism in Neurological and Psychiatric Disorders

"The major focus our study is to understand how the neurotransmitter energetics (Neuronal and astroglia metabolic activity, and their interaction) is manifested in different neurodegenerative (Alzheimer's disease, Amyotrophic Lateral Sclerosis, Parkinson disease) and neuropsychiatric disorders (Depression, addiction)"

Selected recent publications

- Mishra PK, Kumar A, Behar KL, Patel AB* (2018). Sub-Anesthetic Ketamine Reverses Neuronal and Astroglial Metabolic Activity Deficits in a Social Defeat Model of Depression. *Journal of Neurochemistry* 146: 722-734.

- Patel AB*, Tiwari V, Veeraiah P, Saba K (2018). Increased Astroglial Activity and Reduced Neuronal Function Across Brain in AβPP-PS1 Mouse Model of Alzheimer's Disease. *Journal of Cerebral Blood Flow & Metabolism* 38: 1213-1226.

- Veeraiah P, Noronha JM, Maitra S, Bagga P, Khandelwal N, Chakravarty S, Kumar A, Patel AB* (2014). Dysfunctional Glutamatergic and GABAergic Activities in Prefrontal Cortex of Mice in Social Defeat Model of Depression. *Biological Psychiatry* 76: 231-238.

- Patel AB*, Lai JCK, Chowdhury GMI, Hyder F, Rothman DL, Shulman RG, Behar KL (2014). Direct Evidence for Activity-Dependent Glucose Phosphorylation in Neurons With Implications for the Astrocyte-to-Neuron Lactate

Shuttle. *Proceedings of the National Academy of Sciences of the United States of America* 111: 5385–5390.

- Tiwari V, Ambadipudi S, Patel AB* (2013). Glutamatergic and GABAergic TCA Cycle and Neurotransmitter Cycling Fluxes in Different Regions of Mouse Brain. *Journal of Cerebral Blood Flow & Metabolism* 33: 1523-31.

The major emphasis of the group is to understand neurotransmitter eneregetics in different neurological and psychiatric disorders such as Alzheimer's disease, amyotrophic lateral sclerosis, depression and addictions. To achieve this goal, we use stable ^{13}C tracer approach, which involves infusion of ^{13}C labeled substrates (glucose, acetate, etc) in mice, and measure labeling of neurometabolites in brain. The oxidation of [1,6-$^{13}C_2$]glucose in brain incorporates label into glutamate-C4 (Glu_{C4}) and GABA-C2 ($GABA_{C2}$). The labeling of glutamine-C4 (Gln_{C4}) occurs by trafficking of ^{13}C labeled neurotransmitters into astrocytes. The selective oxidation of [2-^{13}C] acetate into astrocytes labels Gln_{C4}, followed by Glu_{C4} and $GABA_{C2}$ via neurotransmitter cycling. Therefore, amount of ^{13}C label trapped into neurometabolites provides a quantitative estimate of metabolic activity of neurons and astrocytes, and neurotransmitter cycling in the brain. We use ^{1}H-[^{13}C]-NMR spectroscopy to quantify ^{13}C label incorporated into different brain regions. This approach is being exploited to understand the etiology of Amyotrophic lateral sclerosis (ALS), depression and Alzheimer's disease (AD), and impacts of various interventions on metabolic changes associated with these disorders. These measurements are supported by behavioral measures in animals.

Amyotrophic Lateral Sclerosis (ALS) is the most common adult-onset progressive motor neuron degeneration disorder, characterized by skeletal muscle atrophy. Despite several years of intensive research no reliable biomarker for diagnosis or prognosis of ALS is available till date. One of the major focuses of the group is to understand the pathophysiology of ALS using transgenic $SOD1^{G37R}$ mouse model. Earlier, we have shown that female $SOD1^{G37R}$ mice exhibit reduced levels of glutamate, NAA, NAAG, and decreased metabolic activity in glutamatergic and GABAergic neurons in the spinal cord at the age of 12 months. In contrast, neurometabolic activity was increased in the cerebral cortex of these mice. Moreover, the astroglial metabolic activity was enhanced suggesting microgliosis in the spinal cord. However, there was no change in neural activity in the brain stem. In continuation of the study, we have evaluated behavior and neurometabolic activity at the age of 18 months. The forelimb strength of $SOD1^{G37R}$ mice was decreased significantly when compared with wild type controls. Additionally, in accordance with 12 month age, the metabolic activity of glutamatergic and GABAergic neurons were reduced in the spinal cord of female $SOD1^{G37R}$ mice. It should be noted that the reduction in the forelimb strength and neurometabolic activity was higher in the aged mice.

To understand the role of gender in the etiology of ALS, we performed metabolic analysis in male $SOD1^{G37R}$ mice. There was no change in the forelimb strength and neurometabolic activity in the central nervous system at 6 month in $SOD1^{G37R}$ mice.The transgenic male $SOD1^{G37R}$ mice exhibited hyper-neurometabolic activity in the cerebral cortex at 12 month age when compared with the age matched wild type controls, similar to that observed in female mice of same age (Fig. 1). However, there was no perturbation in the neurometabolic in other brain regions. Additionally, these mice exhibit increased acetate oxidation in the cerebral cortex and spinal cord. Impairment in motor function, and neurometabolic activity is also observed in the cerebral cortex at the age of 15 months (Fig. 1). There was no significant change in the neurometabolism in the brainstem and spinal cord.

Another major interest of our group revolves around understanding the pathophysiology of Alzheimer's disease (AD) using chemical and transgenic mouse models of the disease. Earlier, we have shown that the neuronal metabolic activity is decreased while astroglial function is increased with progress of AD using transgenic APP-PS1 mice. Moreover,

metabolic changes precede neurodegeneration and clinical symptoms in AD. Similar observations were made using triple transgenic (APP, PS1 & Tau) mice. Moreover, we found that females are less susceptible to AD than males till their reproductive age. Additionally, we have shown that intervention with riluzole, and withania somnifera alleviates memory and neurometabolic activity in APP-PS1 mice. In continuation, we have followed Aβ-plaque loading in APP-PS1 mice with age using immunohistochemical assay. The Aβ-plaques were absent in 3 month old male and female mice. The number and area occupied by Aβ plaques in the cerebral cortex as well as in hippocampus were significantly more in females than males at 6 and 12 month age (Fig. 2A). The increased plaque loading together with no perturbation in memory and brain energy metabolism in female till their reproductive age suggest Aβ-plaque load does not clearly represent the AD pathology.

Although the number of sporadic AD surpass the number of familial cases, most of the studies have used genetic models to understand the AD mechanism. To dissect the mechanism in sporadic AD, we investigated memory and neurometabolism in mice after intracerebroventricular injections of streptozotocin (ICV-STZ, 5mg/kg). These mice spent less time with novel objects suggesting compromised memory in STZ treated mice. There was significant reduction in levels of glutamate, GABA, aspartate and NAA in the cerebral cortex and hippocampus of STZ mice. In contrast, level of myo-inositol was increased in the hippocampus. Most interestingly, the amount of ^{13}C label trapped into Glu_{C4}, $GABA_{C2}$, Gln_{C4}, Glu_{C3} and Asp_{C3} from [1,6-$^{13}C_2$]glucose in 10 minutes was reduced significantly, indicating a loss in metabolic activity of Glutamatergic and GABAergic neurons in the cerebral cortex and hippocampus in STZ treated mice (Fig. 2B). These results suggest decreased excitatory and inhibitory neurotransmission associated with glutamatergic and GABAergic neurons in sporadic AD.

In collaboration with Dr. Arvind Kumar, we are investigating pathophysiology of depression using social defeat (SD) and chronic unpredictable mild stress (CUMS) model of depression. We have shown earlier that neuronal and astroglial metabolic activity is reduced in the pre-frontal cortex in SD and CUMS models of depression. In continuation of these studies, we discovered that pentose phosphate flux is reduced in the cortical and sub-cortical regions suggesting a weakening in anti-oxidant defense system of neural cells in depression. Additionally, we discovered that supplementation of Rasa Sindoor, a formulation used in Indian traditional medicine, maintained the behavioral phenotype and neurometabolism in CUMS mice to control level. Moreover, we have shown that acute sub-anesthetic dose of ketamine reverses the behavioral and metabolic deficits in SD mice. Very recently, we have shown that few doses of lanicemine, a low trapping NMDA channel blocker, also reverses the behavioral and neural metabolic measures in CUMS mice. To understand the mechanism of action of the fast acting antidepressants, the impact of sub-anesthetic ketamine, a NMDA receptor antagonist, on the neurotransmitter metabolism in mice was evaluated. Low dose of ketamine (10 mg/kg, i.p.) did not perturb neurometabolism in the prefrontal cortex in control mice in 15 minutes. However, acute ketamine at 25 mg/kg enhanced metabolic activity of both Glutamatergic and GABAergic neurons in the first 15 min of the administration, and was not significantly different after 25 min of the treatment. It is noteworthy that ketamine (25 mg/kg, i.p.) did not affect neurometabolic activity in the cerebral cortex and hippocampus. In addition, acute ketamine did not affect the astroglial metabolic activity in the prefrontal cortex and hippocampus. Unlike ketamine, acute lanicemine (25 mg/kg, i.p.) did not perturb the neurometabolic activity across the brain in control mice. Further studies are needed to understand the mechanism of fast acting antidepressants.

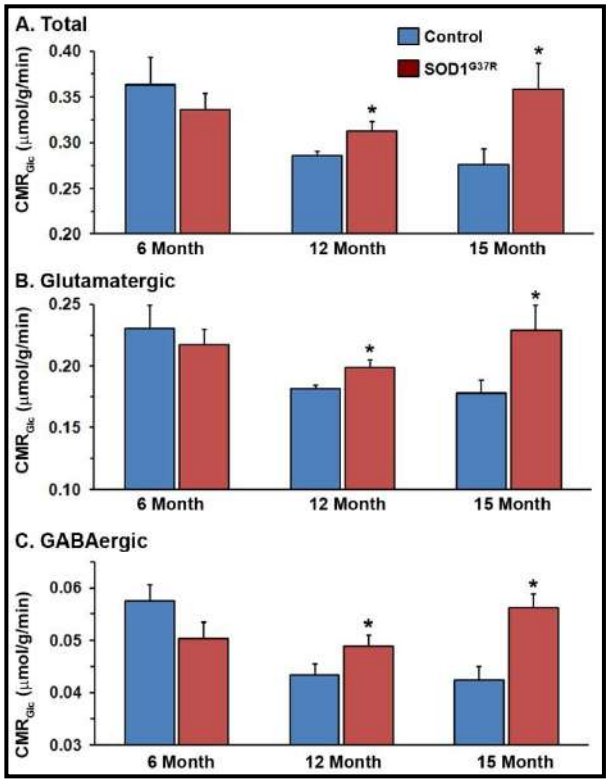


Fig. 1: Cerebral metabolic rates of glucose oxidation with progress of age in male SOD1^{G37R} mice. A. Total, B. Glutamatergic Neurons, and C. GABAergic neurons.

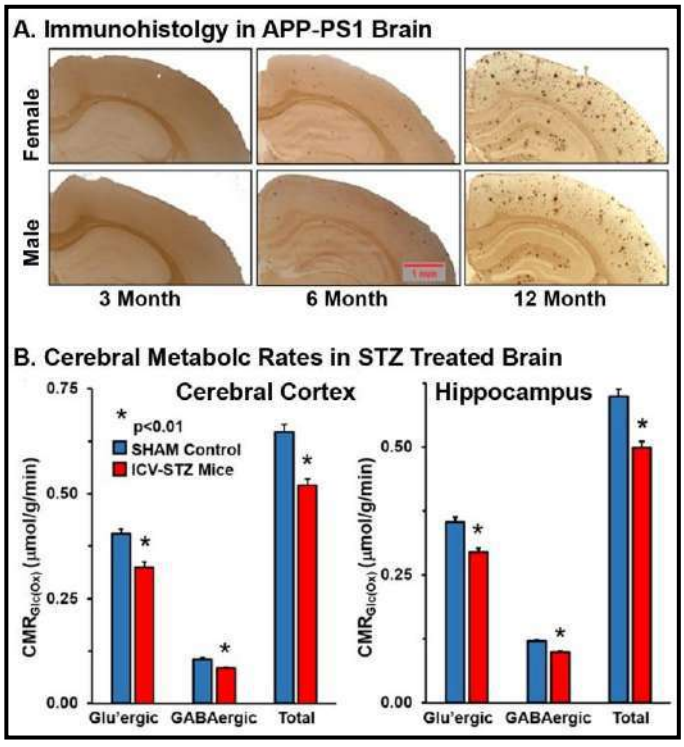


Fig. 2A: Immunohistogical images depicting Aβ plaque load with age in male and female APP-PS1 mice. **B.** Cerebral metabolic rates of glucose oxidation in streptozotocine treated mice.

R NAGARAJ

Host-defense Antimicrobial Peptides; Activity and Developing Future Therapeutic Agents

From left to right: R. Nagaraj, Ankeeta Guru, Taniya, Tushar Ranjan

RESEARCH INTERESTS:

- Host-defense antimicrobial peptides: various facets of their biology
- Peptide nanostructures: Their formation, structure and applications.

"Self-assembly of engineered amyloid peptides. Effects of introducing beta turn initiating dipeptides in amyloidogenic peptides."

Selected recent publications

- Datta D, Harikrishna A, Nagaraj R, Chaudhary N (2018). Self-assembly of β-turn motif-connected tandem repeats of Aβ16-22 and its aromatic analogs. *Peptide Science* doi.org/ 10.1002 /pep2.24099
- Yadav K, Rao JL, Srinivas R, Nagaraj R, Jagannadham MV (2018). Characterization of acetylated histidine b1-ion structure: A competition between oxazolone and side chain imidazole moiety. *European Journal of Mass Spectrometry* 24: 261-268.
- Krishnakumari V, Guru A, Adicherla H, Nagaraj R (2018). Effects of increasing hydrophobicity by N-terminal myristoylation on the antibacterial and hemolytic activities of the C-terminal cationic segments of human-β-defensins 1-3. *Chemical Biology and Drug Design* 92: 1504-1513.
- Jagannadham MV, Kameshwari DB, Gayathri P, Nagaraj R (2018). Detection of peptides with intact phosphate groups using MALDI TOF/TOF and comparison with the ESI-MS/MS. *European Journal of Mass Spectrometry* 24: 231-242.
- Datta D, Kumar V, Kumar S, Nagaraj R, Chaudhary, N (2019). Hydrogel Formation by an Aromatic Analogue of

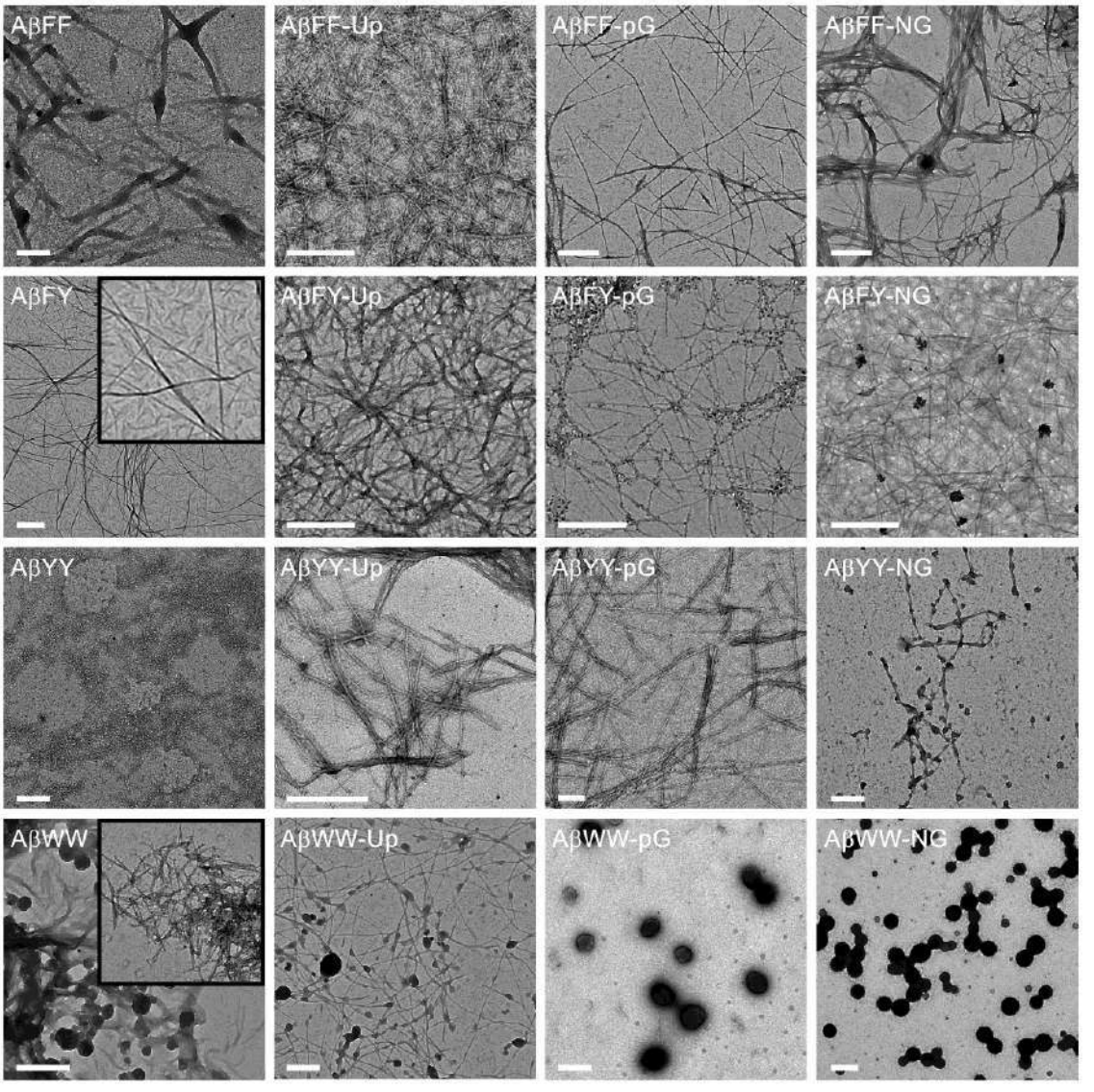
a β-Amyloid Fragment, Aβ16–22: A Scaffold for 3D Cell Culture. *American Chemical Society Omega* 4: 620-627.

Self-assembly of β-turn motif-connected amyloid peptides

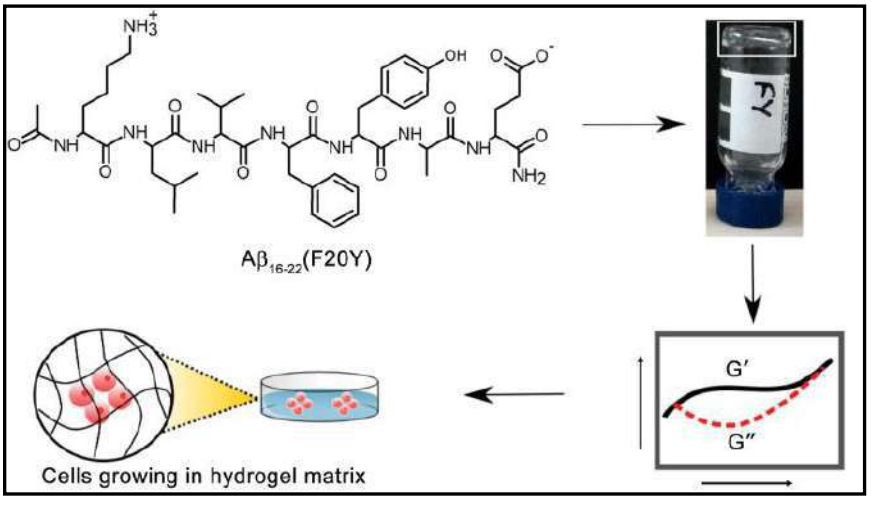
Self-assembly of peptides and proteins into aggregates with a signature of cross-β conformation is a hallmark of amyloids. Short peptides have provided important insights in understanding the various interactions that drive self-assembly as well as the molecular architecture of the self-assembled structures. The short amyloidogenic-stretch of β-amyloid, Aβ16-22 (Ac-KLVFFAEam) is a good model peptide to study the aspects of β-amyloid fibril formation. In order to investigate how a turn-supporting sequence could modulate the interaction of the AcKLVXZAE-am chains, where X and Z are the aromatic amino acids, Phe, Tyr, or Trp, we investigated the self-assembly of Ac-KLVFFAE-am, Ac-KLVFYAE-am, Ac-KLVYYAE-am, and Ac-KLVWWAE-am separated by turn-inducing dipeptide motifs, Asn-Gly, DPro-Gly, and Aib-DPro. The peptides harboring β-turn-inducing motifs aggregate rapidly causing large enhancements in ThT fluorescence compared to control, β-turn motif lacking peptides. The morphology of fibrils strongly depends on the type of β-turn. Ac-KLVFYAE-am repeats separated by Aib-DPro and DPro-Gly have the highest aggregation propensity among all the peptides studied; they caused very large enhancement in ThT fluorescence. Ac-KLVYYAE-am is largely non-amyloidogenic; the DPro-Gly and Aib-DPro connected repeats, however, resulted in distinct fibrils that bind ThT. The study indicates that β-turn motifs can be exploited to modulate and control the aggregation propensity of peptides and the morphology of aggregates.

Hydrogel formation by an aromatic analog of β-amyloid fragment, Aβ16-22 – a scaffold for 3-D cell culture

The short 7-residue stretch from β-amyloid, Aβ16-22 (Ac-KLVFFAE-am) yields typicalamyloid-like fibrils at neutral pH. The Phe-Phe cassette present in the peptide is believed to be critical for its self-assembly. We report that the aromatic analog, Aβ16-22(F20Y) forms self- supporting soft gels at concentrations ≥2 mM even though the end-capped parent peptide does not form hydrogel up to 20 mM (1.8% w/w) concentration. The hydrogel is made up of distinct amyloid-like fibers. The storage modulus of 20 mM gel is ~3-5-fold higher than loss the modulus in the 2–3000 rad/s angular frequency range, indicating distinct elastic properties. The hydrogel supports the growth of rat pancreatic cells (RIN-5F), human embryonic kidney cells (HEK-293), baby hamster kidney cells (BHK-21), and human neuroblastoma cells (IMR-32). The cells grow in clusters as is anticipated in a three- dimensional matrix. The rat pancreatic cells produced insulin suggesting that they are functional inside the gel. This study shows that the native amyloidogenic peptides hold the promise to be developed into non-toxic hydrogelators through subtle modifications. Peptide hydrogels formed by short Abeta peptides have potential application in regenerative medicine and slow release of drugs.



TEM analyses of Abeta turn containing peptides. The scale bars represent 0.5 µm



Hydrogel formation of Abeta peptide

SWASTI RAYCHAUDHURI

Proteotoxicity in Age-related Diseases

From left to right: Suparna Ghosh, Shivali Rawat, Swasti Raychaudhuri, Debodyuti Mondal, Harshit Vaish, Shemin Mansuri, Dhanishta, Kiranmayi, Richa Singh, P. Vijaya

RESEARCH INTERESTS:

- Toxic and beneficial outcome of protein aggregation inside cells
- Proteostatic control on respiratory complex biogenesis
- Causes of protein-misfolding diseases being prevalent during aging

"Inside a cell, numerous proteins interact with each-other to form a 'society'; the so-called 'cellular proteome'. Such 'protein-societies' are responsible for the proper functioning of every cell. Any deviation from the functional conformation/concentration of a 'member-protein' may negatively affect the organization of the 'society' and lead to gradual functional impairment. This is called 'proteotoxic-collapse' and is often true for many age-related diseases. Our group is interested in understanding the coordination of the proteins towards a functional proteome and the defence-mechanisms in the face of proteotoxic events"

Selected recent publications

- Saleh A, Subramaniam G, Raychaudhuri S, Dhawan J (2019). Cytoplasmic sequestration of the RhoA effector mDiaphanous1 by Prohibitin2 promotes muscle differentiation. *Scientific Reports* 9: 8302.
- Rawat S, Anusha V, Jha M, Sreedurgalakshmi K, Raychaudhuri S (2019). Aggregation of Respiratory Complex Subunits Marks the Onset of Proteotoxicity in Proteasome Inhibited Cells. *Journal of Molecular Biology* 431: 996-1015.
- Moharir SC, Bansal M, Ramachandran G, Ramaswamy R, Rawat S, Raychaudhuri S, Swarup G (2018). Identification of a splice variant of optineurin which is defective in autophagy and phosphorylation. *Biochimica et Biophysica Acta - Molecular Cell Research* (11 Pt A): 1526-1538.
- Raychaudhuri S, Loew C, Koerner R, Pinkert S, Theis M, Buchholz F, Hartl FU (2014). Interplay of Acetyl transferase EP300 and the Proteasome System in Regulating Heat Shock Transcription Factor 1. *Cell* 156: 975-985.

- Gupta R, Kasturi P, Bracher A, Loew C, Zheng M, Villella A, Garza D, Hartl FU*, Raychaudhuri S*. (2011). Firefly luciferase mutants as sensors of proteome stress. *Nature Methods* 8: 879-884. *Corresponding author

Aggregation of Subunits and Biogenesis of Respiratory Complexes

Proper folding and solubility are two major determinants of protein function. Any physicochemical stress that may perturb protein-conformation is capable of triggering protein aggregation. Previous studies have reported that physical stresses can trigger rapid aggregation of many endogenous proteins that are repairable upon withdrawal of stress. Unlike physical and acute forms of proteotoxic stresses, proteostasis damage corresponds to a chronic form of stress that results in continuous accumulation of protein aggregates relevant in many age-related diseases. Despite multiple studies, it remains unknown whether this widespread protein-aggregation process is stochasticin occurrence or initiated by instability of a specific group of proteins, dictated by their physicochemical signatures or cellular function.

Using quantitative proteomics and microscopy we discovered that nuclear-encoded Respiratory Chain Complex (RCC) subunits readily form aggregates when over-accumulate in cytosol due to multiple proteostasis stresses. Intrinsic instability of these subunits is determined by diverse physicochemical-signatures including low complexity regions (LCRs) at N-termini that drive their translocation fostering functional assembly inside mitochondria (Fig.1). Further, we found that formation of diverse higher order Respiratory Compexes (RCs) is favoured over free RCC subunits and sub-complexes to achieve optimum performance in response to proteostasis-stresses. We named this dynamic posttranslational defence mechanism as 'improved Supra-organization of Respiratory Complexes' (iSRC). We observed that this reversible spatiotemporal proteome adaptation mechanism is highly regulated and preserves the most indispensable activity in mitochondria - consistent with a coordinated effort to sustain cellular bioenergetics to survive proteotoxicity. Controlled and specific association between proteins preserves solubility, essential for a myriad of biological functions. iSRC represents an example where this fundamental principle is exploited to adapt to proteostasis stress even before triggering quinary associations of RCC subunits that provide passive proteome-insulation against stress.

Proteotoxicity by α-Synuclein

We are using α-Synuclein (SNCA) expressing cell-culture models to understand the mechanism of proteotoxicityin protein-misfolding diseases. Duplications and triplications of *snca* gene as well as mutations in the N-terminal region (A53T, A30P, E46K etc.) cause aggregation of this protein resulting in a range ofsynucleinopathies. We have successfully generated inducible stable-lines in HEK293T cells expressing wild-type α-synuclein (SNCA-wt), mutants A53T, A30P and the double mutant (DM; A53T+A30P). We have also purified recombinant wild-type and mutant α-synuclein proteins and compared amyloidosis *in vitro* (preformed amyloid fibrils (PFF)). Upon long-term (7 days) incubation with these PFF, cells expressing EGFP tagged SNCA-wt and A30P showed diffuse EGFP fluorescence whereas SNCA-A53T and DM showed EGFP-positive hairy aggregates. These results suggest that the *in vitro* generated PFF crossed the plasma membrane barrier; or caused substantial damages in the cytoplasmic proteome via altering membrane-trafficking that resulted in aggregation of EGFP-tagged endogenous SNCA-A53T and SNCA-DM (Fig. 2).

Questions being addressed

a) This model opens up the opportunity to study the alteration of “membrane proteome” and follow up cell biology investigations to provide knowledge on SNCA fibril-entry mechanism into the cells.
b) Differences in the amyloid-formation kinetics between the SNCA-variants indicate presence of diverse seeding-intermediates in the cells. During PFF incubation, the folding-pattern of the overall-proteome is expected to be changed depending on the seeding-intermediates and with time. We

are performing experiments to characterize the differences between the protein-folding patterns in these different stages. The improvised proteomics experiments will provide mechanistic knowledge on the propagation of proteotoxicity during amyloidosis.

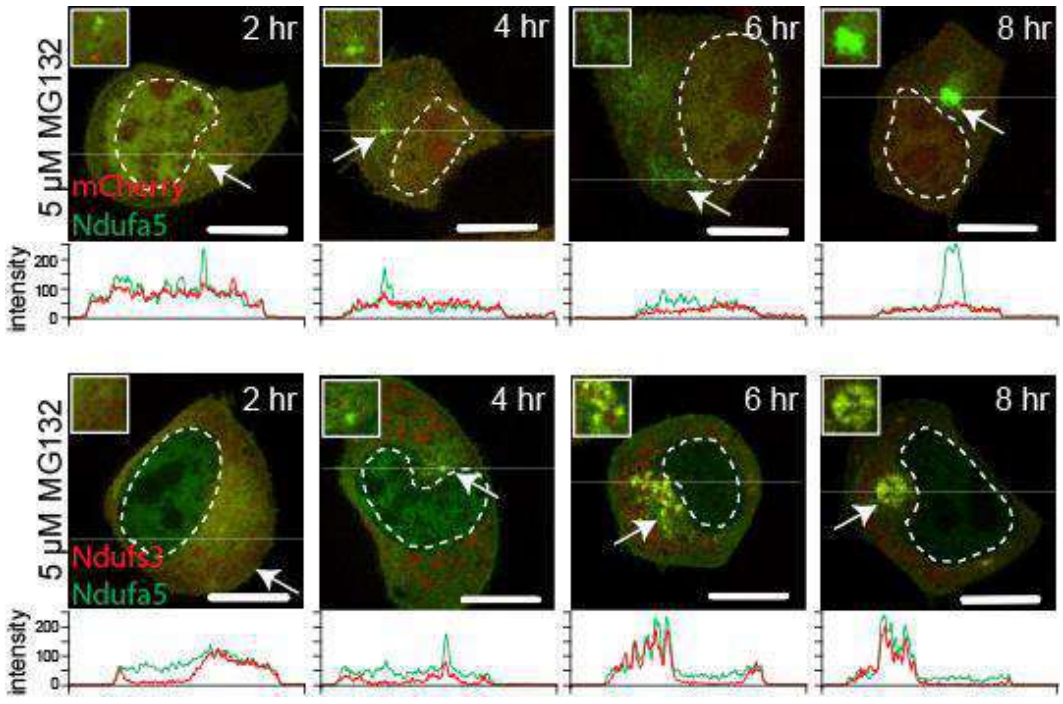


Fig. 1: Microscopy images of Ndufa5-EGFP co-transfected with mCherry or Ndufs3-mCherry. Cell were treated with MG132 for indicated time points. Cell fixation was performed using paraformaldehyde. Fluorescence intensity profile along the line is shown. Nucleus is shown by dotted lines. Arrows indicate IBs shown in zoomed insets. Scale-bar - 10 µm.

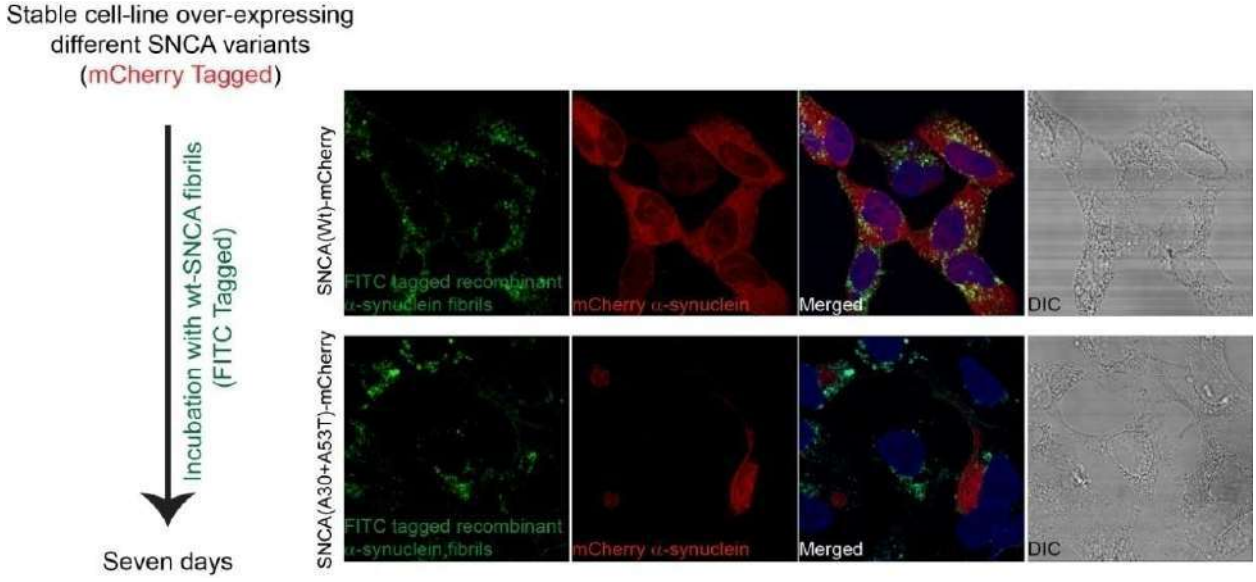


Fig. 2: Uptake of FITC-tagged Pre-formed Fibrils (PFF) by HEK293T cells expressing Wild type mCherry-tagged α-synuclein and the double mutant (A30P+A53T) and formation of chimeric aggregates. Cells were incubated with FITC tagged PFF for seven days. Fluorescence microscopy images show presence of PFF inside the cells (green) and aggregation of endogenous synuclein (red).

MANJULA REDDY

Bacterial Cell Wall Synthesis and its Regulation

From left to right: GSN Reddy, Shambhavi Garde, Manjula Reddy, Moneca Kaul, S Venugopal, Nilanjan Som, Richa Khanna, Pavan Kumar Ch, Raj Bahadur, Balaji Venkataraman

RESEARCH INTERESTS:

- Understanding bacterial cell wall synthesis and its regulation

"Research in my laboratory is focused towards understanding how bacteria elongate, divide and split their cell walls during cell cycle to successfully generate two equal daughter cells. We take a multi-disciplinary approach including genetics, biochemistry, cell biology, and genomics to address these questions using a Gram-negative rod-shaped bacterium *Escherichia coli* as primary model system"

Selected recent publications

- Chodisetti PK, Reddy M (2019). Peptidoglycan hydrolase of an unusual cross-link specificity contributes to bacterial cell wall synthesis. *Proceedings of National Academy of Sciences USA* 116: 7825-7830.
- Mychack A, Amrutha RN, Chung C, Cardenas AK, Reddy M*, Janakiraman A*(2019). A synergistic role for two predicted inner membrane proteins of *Escherichia coli* in cell envelope integrity. *Molecular Microbiology* 111: 317-337. (*Co-corresponding)
- Parveen S, Reddy M (2017). Identification of YfiH (PgeF) as a factor contributing to the maintenance of bacterial peptidoglycan composition. *Molecular Microbiology* 105: 705-720.
- Singh SK, Parveen S, SaiSree L, Reddy M (2015). Regulated proteolysis of a crosslink-specific peptide glycan hydrolase contributes to bacterial morphogenesis. *Proceedings of National Academy of Sciences USA.* 112: 10956-10961.

- Mahalakshmi S, Sunayana MR, SaiSree L, Reddy M(2014). *yciM* is an essential gene required for regulation of lipopolysaccharide synthesis in *Escherichia coli*. *Molecular Microbiology* 91: 145-157.

Our research facilitates better understanding of fundamental aspects of bacterial cell biology and we expect that our work will provide novel strategies for development of antimicrobial therapeutics.

Bacteria are a large group of ubiquitous unicellular microorganisms. To protect cells against both external and internal osmotic pressure, the bacterial cell envelopes contain an essential and unique cage-like molecular sieve called peptidoglycan (PG or murein) sacculus. Structurally, PG is made up of multiple overlapping glycan strands with short peptide chains that are cross-bridged to each other resulting in a net-like single- or multilayered sacculus. PG completely encases the bacterial cytoplasmic membrane (or inner membrane, IM) and hence gives a characteristic shape to bacteria. In Gram-negative organisms, the PG sacculus is enclosed by an additional bilayered lipid membrane, the outer membrane (OM) that restricts the entry of large hydrophobic molecules into the cells. Collectively, these three layers of bacteria are referred to as 'cell wall' or 'cell envelope'. During cell cycle progression, this complex cell wall poses a challenge because all the three layers (i.e., the OM, the PG sacculus and the IM) must coordinately grow in length during elongation phase (as cell expands in size and volume) and also invaginate to form a septum during division phase for successful generation of daughter progeny. To understand this fundamental process, we chose to use *Escherichia coli*, a Gram-negative rod as a model system because it is one of the best studied and genetically amenable bacterium with readily available resources and reagents.

Work done earlier

Of the three layers of the Gram-negative cell envelope, the enlargement of PG sacculus during cell elongation is a challenging task as PG is an extensively cross-linked large macromolecule that completely encases the IM. Therefore, as a cell expands in size, the PG sacculus is also expected to grow and enlarge. However, to enlarge such a covalently cross-linked molecule, the interconnecting cross-bridges between the glycan chains need to be broken for incorporation of new murein material (Fig. 1). We earlier identified three murein hydrolytic enzymes, two (MepS, MepH) belonging to the NlpC/P60 peptidase superfamily and the third (MepM) to the lysostaphin family of proteins that cleave peptide cross-bridges between glycan chains. We showed that these endopeptidases are redundantly essential for bacterial growth and viability as a conditional mutant lacking all the three enzymes is unable to incorporate new murein and undergoes rapid lysis upon shift to restrictive conditions. Of the three endopeptidases identified, we demonstrated that expression of MepS is stringently regulated at the level of post-translational stability indicating that PG hydrolysis is the rate-limiting step of PG enlargement.

Additionally, our studies delineated a new regulatory pathway for the synthesis of LPS (lipopoly saccharide), which forms the outermost leaflet of OM. We identified and showed that *yciM*, a gene encoding a tetratricopeptide repeat protein of unknown function, modulates LPS levels by negatively regulating the biosynthesis of lipid A, an essential constituent of LPS. YciM regulates LPS by altering LpxC, a rate-limiting enzyme that catalyzes the first committed step of lipid A biosynthesis and this regulation was contingent on the presence of FtsH, an essential membrane-anchored protease known to degrade LpxC.

Work done during the year

The glycan chains of bacterial PG are cross-linked through short peptide chains by two types of linkages: the predominant linkages are between the D-alanine and *meso*diaminopimelic acid (D-ala–mDAP) and the rare linkages are between the mDAP and mDAP residues (mDAP–mDAP). Although, we have earlier shown the importance of D-ala–mDAP cross-link cleavage in PG expansion, the significance of mDAP–mDAP cross-link cleavage in PG synthesis was not clear. During the year, our efforts have led to identification of a previously unknown open reading

frame, YcbK (redesignated Murein endo peptidase K, MepK) as a PG hydrolase that is specific to hydrolysis of mDAP–mDAP cross-links. Deletion of *mepK* was done along with deletion of *mepS*that codes for a major D-ala–mDAP specific endopeptidase; absence of L,D-transpeptidase activity (encoded by *ldtD*, *-E* that catalyze the formation of mDAP–mDAP cross-bridges) was able to abrogate the defects of *mepKmepS* double mutant indicating *ldtD, -E* were epistatic to *mepK*. Detailed genetic and molecular analyses further confirmed that MepK works in conjunction with other D-ala–mDAP specific endopeptidases to contribute to growth and synthesis of PG. Purified MepK was able to specifically cleave the mDAP–mDAP cross-links of soluble muro peptides and of intact PG sacculi. MepKorthologs from other Gram-negative bacteria also exhibited similar substrate-specificity indicating a functional conservation across bacterial genera (Chodisetti and Reddy, PNAS 2019).

In a few bacteria including *E. coli*, the PG sacculus is tethered to OM by an abundant lipoprotein called Braun's lipoprotein or Lpp. Three L, D-transpeptidases, LdtA, -B, -C catalyze the formation of a cross-link between the extreme C-terminal amino acid, lysine of Lpp and an mDAP residue of a peptide thereby anchoring the PG to the OM. We recently identified a novel PG hydrolase that cleaves Lpp from the PG sacculus of *E. coli*. Using genetic and biochemical approaches, we demonstrate that this endopeptidase cleaves the Lys-mDAP linkages in both soluble muropeptides and intact PG sacculi suggesting this endopeptidase along with LdtA, -B and -C maintains an optimal OM-PG tethering contributing to the structural integrity of the cell envelope (manuscript in preparation).

In addition, we studied the mechanistic basis of LpxC regulation by YciM and FtsH. We showed that YciM senses the acylation status of the cell by binding to the acylated-Acyl Carrier protein (acyl-ACP; holoenzyme) and directs FtsH protease to degrade LpxC, the rate-limiting enzyme in lipid A biosynthesis thereby controlling the levels of LPS in the OM (Balaji and Reddy, manuscript in preparation).

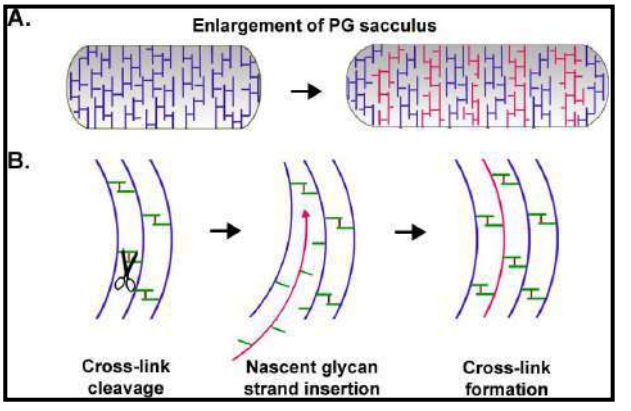


Fig. 1: Schematic of PG enlargement. A. Depiction of PG enlargement during elongation-phase. Red indicates newly incorporated glycan strands and blue are preexisting glycan strands. B. Cleavage of cross-links is followed by resynthesis for successful expansion of PG layer. Green and red bars represent peptide chains and cross-bridges between them, respectively

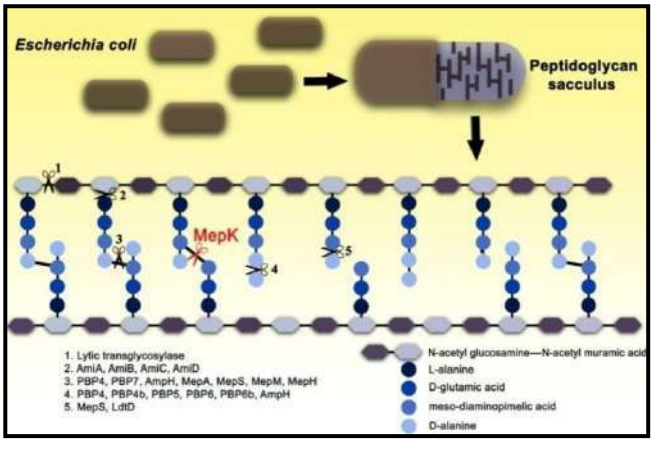


Fig. 2: Schematic of *E. coli* peptidoglycan depicting the cleavage sites of various PG hydrolytic enzymes.

KUMARASWAMY REGALLA

Cardiovascular Biology

From left to right: Kumaraswamy, Priyanka Pant, Disha Nanda, Abhishek Bharadwaj

RESEARCH INTERESTS:

- Non-coding RNAs and heart failure
- Animal models of cardiovascular diseases

"Increasing evidence suggests that non-coding RNAs (micro-RNAs and long non-coding RNAs) regulate myriad biological processes. In our lab, we study the role of non-coding RNAs in cardiovascular diseases such as heart failure and aneurismal disease"

Selected recent publications

- Adelsperger AR, et al. (2018). Development and growth trends in angiotensin II-induced murine dissecting abdominal aortic aneurysms. *Physiological Reports* 6: 1-17.
- Hong Lu, Aikawa M (2015). Many Faces of Matrix Metalloproteinases in Aortic Aneurysms. *Arteriosclerosis, Thrombosis, and Vascular Biology* 35: 752-754.
- Shen M, et al. (2015). Divergent roles of matrix metalloproteinase 2 in pathogenesis of thoracic aortic aneurysm. *Arteriosclerosis, Thrombosis, and Vascular Biology* 35: 888-898.
- Cheng J, Koenig SN, Kuivaniemi HS, Garg V, Hans CP (2014). Pharmacological inhibitor of Notch signaling stabilizes the progression of small abdominal aortic aneurysm in a mouse model. *Journal of the American Heart Association* 3: 1-26.
- Zheng YH, et al., (2013). Notch γ-secretase inhibitor dibenzazepine attenuates angiotensin II-induced

abdominal aortic aneurysm in ApoE knockout mice by multiple mechanisms. *PLoS One* 8: 1-14.

- Gwathmey JK, et al. (1987). Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. *Circulation Reseach* 61: 70-76.

Role of a specific long non-coding RNA in aortic aneurism

Aortic Aneurysms are life threatening vascular complications that manifest as local dilations of the aorta which can rupture and result in hemorrhage. Abdominal aortic aneurysms (AAAs) are described as local dilations of the abdominal aorta. Annually, about 150,000 deaths worldwide are attributed to AAAs (Adelsperger et al. 2018). AAA is a vascular complication involving dysfunction of the blood vessel cells including Endothelial cells (ECs) and Vascular Smooth muscle cells (VSMCs). Aortic aneurysms involve weakening of aortic wall and compromised integrity of extracellular matrix (ECM). Among many ECM proteins, Elastin provides recoil property to the aorta and Collagen provides tensile strength to withstand high blood pressure during cardiac systole. Physiological turnover of ECM is mediated by degradation of these ECM proteins through matrix metalloproteinases (MMPs) and synthesis of new ECM proteins. MMP2 and MMP9, which are gelatinases have been linked with aneurysm development as these can degrade Elastin and Collagen. Several studies have found elevated levels of MMP2 in aneurismal aortas (Hong Lu et al, 2015 and Shen et al 2015) and also during cardiac fibrosis. Recently, protein expression profiling studies revealed that Notch signalling is highly activated in aneurismal aorta. Pharmacological inhibition of Notch signaling by γ-secretase inhibitor attenuated development of AAA in mouse model (Cheng et al, 2014 and Zhent et al 2013). Notch signaling is already known to induce MMP2 and MMP9. Preliminary evidence from our lab has indicated that a Notch regulated lncRNA (L213, internal reference), is up regulated in VSMCs when stimulated with aneurysm inducing agent Angiotensin II. Up regulation of MMP2 levels upon Ang II treatment paralleled with activation of Notch signalling(as verified by up regulation of classical Notch responsive genes like Hey1 and Jagged1). Under these conditions, inhibition of Notch signalling by treating cells with γ-secretase inhibitor DAPT blunted angiotens in II induced Notch activation, lncRNAL213 and MMP2 induction (Fig 1B).

We are now performing *in vitro* and *in vivo* loss of function studies to ascertain the role of this lncRNA candidate L213 in AAA.

Long non-coding RNAs and heart failure

Compromised contractility is one of the most prominent features of end-stage heart failure and this reduction in contractility is due to disturbances in calcium signalling[17]. One of the key proteins involved in this maintenance is SERCA (sarcoendoplasmic reticulum (SR) calcium transport ATPase) which is a P type ATPase located on sarcoplasmic membrane. At the end of each contraction, SERCA pumps uptake the calcium from cytosol and aids in its sequestration in SR until the next contraction cycle. It is well established that expression of cardiac specific isoform, SERCA2a (sarcoendoplasmic reticulum (SR) calcium transport ATPase, type2a) is decreased at the transcript and protein level in patients with end-stage heart failure (Gwathmeyet al, 1987). In our lab, we found that pharmacological inhibition of SERCA by thapsigargin results in elevation of endoplasmic reticulum stress and up regulation of lncRNAL72 in rat cardiomyoblast cell line H9C2 (Figure 2). Similarly, treatment of H9C2 with pro-hypertrophic agent isoproterenol resulted in up regulation of cardiac stress markers ANP and BNP along with upregulation of L72 and its transcript variant L72.1 Figure 2b). We are presently investigating the role of lncRNA L72 in cardiac hypertrophy.

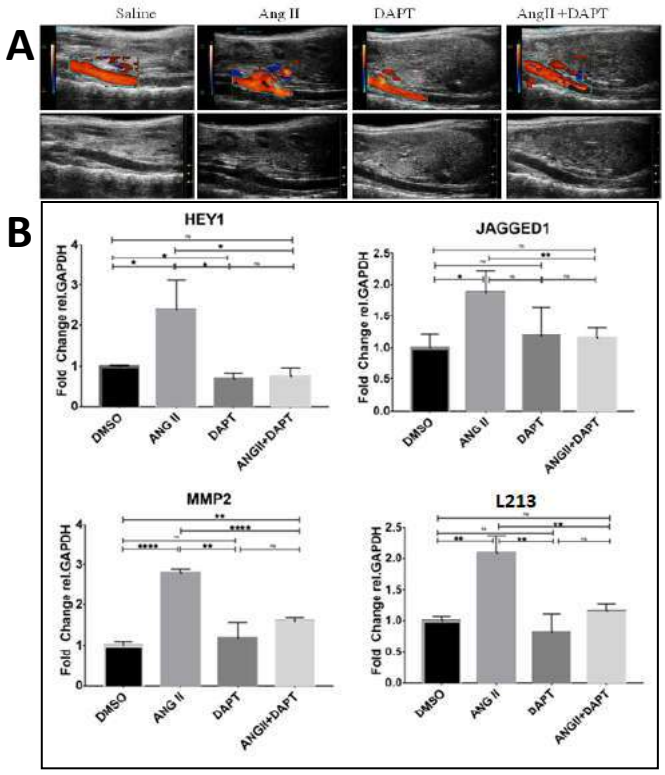


Fig. 1: (A) Attenuation of Ang II induced Abdominal Aortic Aneurysm (AAA) by Notch inhibition: Angiotensin II was intravenously infused in Apo E-/- mice for 56 weeks. A sub-group of Ang II infused animals were also treated with Notch inhibitor DAPT. Note induction of AAA by Ang II and its attenuation by Notch inhibition. **(B)** Ang II induced induction of MMP2 up regulation coincides with Notch activation and lncRNAL213 over-expression.

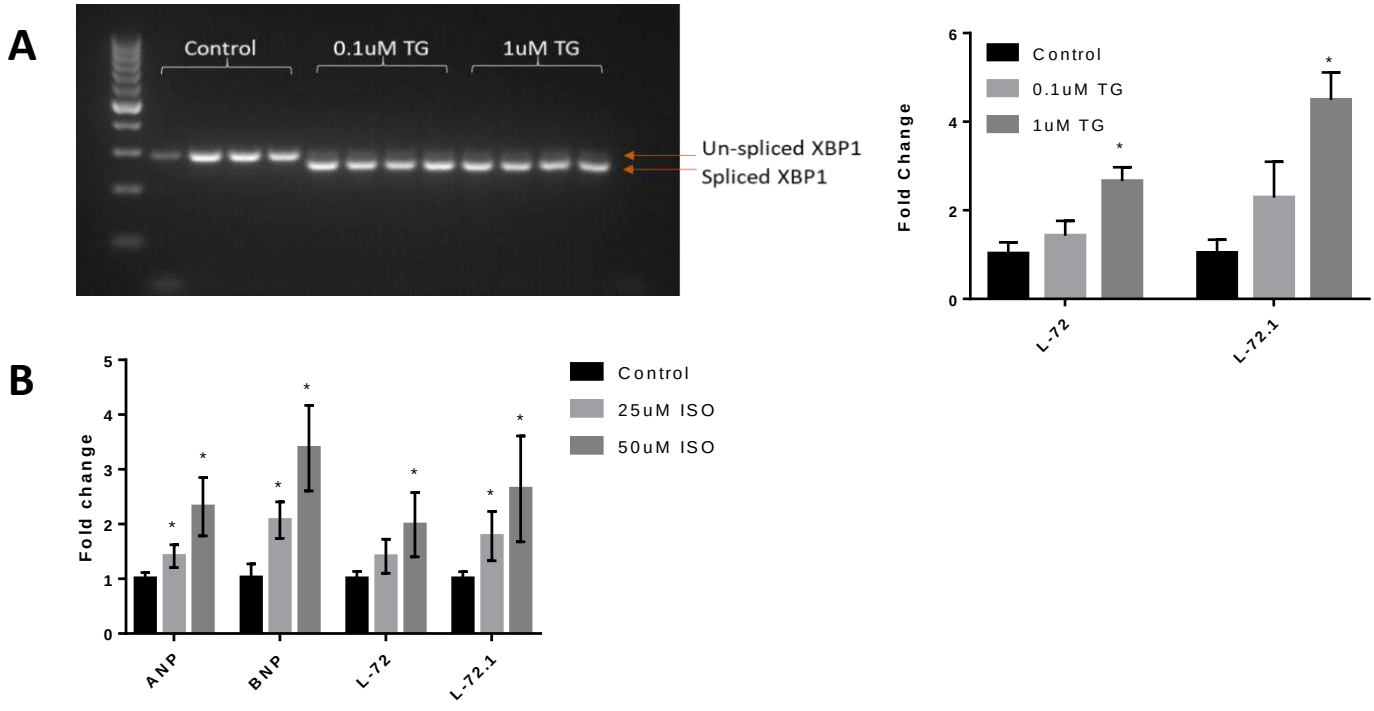


Fig. 2: (A) Inhibition of SERCA using thapsigargin in rat cardiomyoblast cell line H9C2 resulted in ER stress as evident from XBP1 mRNA splicing, which coincides with up regulation of L72 and L72.1 **(B)** Treatment of H9C2 cells with prohypertrophic agent isoproterenol resulted in upregulation of cardiac stress markers ANP and BNP and lncRNA L72 and L72.1.

RAJAN SANKARANARAYANAN

Structural Biology

From left to right: Puspangna Singh, Raghvendra Singh, Rajkanwar Nathawat, Aditya Jamkhindikar, Sujatha, Pradeep Kumar, Noopur Dubey, Gajanan Shrikant Patil, R. Rukmani, Rajan Sankaranarayanan, Lalitha, Santosh Kumar Kuncha, Surabhi Pramanik, Shobha Krupa Rani, Gurumoorthy A, Mazeed Mohd, Vinita Lakshmi V, Kezia J Ann, Jotin Gogoi, Priyanka Dahate, Biswajit Pal, Aravind, Sambhavi Pottabathini, K Mallesham, Sudipta Mondal, Ankit Roy, Priyadarshan K, Akshay Bhatnagar

RESEARCH INTERESTS:

- Proofreading during translation of the genetic code
- *Mycobacterium tuberculosis* complex lipid synthesis

"Our principal focus is to elucidate various proofreading/editing mechanisms involved in the maintenance of quality control during translation of the genetic code as well as to understand their physiological significance."

Selected recent publications

- Kuncha SK, Suma K, Pawar KI, Gogoi J, Routh SB, Pottabathini S, Kruparani SP, Sankaranarayanan R (2018). A discriminator code-based DTD surveillance ensures faithful glycine delivery for protein biosynthesis in bacteria. *Elife* 7: e38232, 1-15.
- Kuncha SK, Mazeed M, Singh R, Kattula B, Routh SB, Sankaranarayanan R (2018). A chiral selectivity relaxed paralog of DTD for proofreading tRNA mischarging in Animalia. *Nature Communications* 9: 511, 1-13.
- Pawar KI, Suma K, Seenivasan A, Kuncha SK, Routh SB, Kruparani SP, Sankaranarayanan R (2017). Role of D-aminoacyl-tRNA deacylase beyond chiral proofreading as a cellular defense against glycine mischarging by AlaRS. *Elife* 6: e24001, 1-19. (Covered by a Spotlight article in TIBS, September 2017, vol. 42; p684).

- Routh SB, Pawar KI, Ahmad S, Singh S, Suma K, Kumar M, Kuncha SK, Yadav K, Kruparani SP, Sankaranarayanan R (2016). Elongation factor Tu prevents misediting of Gly-tRNA^{Gly} caused by the design behind the chiral proofreading site of D-aminoacyl-tRNA deacylase. *PLoS Biology* 14: e1002465, 1-22.
- Ahmad S, Muthukumar S, Kuncha SK, Routh SB, Yerabham AS, Hussain T, Kamarthapu V, Kruparani SP, Sankaranarayanan R (2015). Specificity and catalysis hardwired at the RNA–protein interface in a translational proofreading enzyme. *Nature Communications* 6*:* 7552.

We exploit X-ray crystallography tools along with biochemical and biophysical approaches to obtain atomic-level structural details of various proteins, which in turn help in gaining mechanistic insights into their functioning. We also perform experiments in various model organisms to investigate the physiological relevance of some of these processes. One of the main focuses of the lab is to understand various factors which are involved in proofreading during translation of the genetic code. In cells, D-amino acids are occasionally mischarged on tRNAs causing mis-translation. D-aminoacyl-tRNA deacylase (DTD) is an enzyme which specifically hydrolyzes the ester bond between D-amino acid and the tRNA, thereby acting as a chiral checkpoint and contributing to the homochirality of the cellular proteome. Structural and biochemical studies have revealed that DTD operates via L-chiral rejection mechanism, which is mainly through the cross-subunit Gly-*cis*Pro motif (GP-motif) which acts as a "chiral selectivity filter". Due to L-chiral rejection, DTD acts on tRNAs charged with achiral glycine (Gly-tRNA$^{Gly/Ala}$), which is beneficial in clearing Gly-tRNA^{Ala}. However, the activity of DTD on non-cognate Gly-tRNA^{Ala} is 1000-fold more compared to that of cognate Gly-tRNA^{Gly}, which clearly suggests the role of tRNA. In the process of identifying the tRNA determinants of bacterial DTD we have uncovered the role of DTD in dictating the discriminator code (N73rd base) of tRNA. Using biochemical studies, we could show that uracil as the discriminator base acts a negative determinant for DTD's action, and is invariant in bacteria tRNA^{Gly}, while this position is usually a purine in rest of the tRNA's which acts as a positive determinant for DTD. Currently we are probing the role of tRNA in modulating DTD's activity in eukaryotes.

Recently we have identified a chiral selectivity relaxed paralog of DTD named as Animalia-specific tRNA deacylase (ATD). Using structural, biochemical, and bioinformatics analysis we have shown that unlike DTD, ATD can also act on tRNAs attached to smaller L-amino acids. The mechanistic basis of this L-amino acid activity is well exemplified in the structure by *cis* (DTD) to *trans* switch of the "chiral selectivity filter" (GP-motif) of ATD. ATD is present in the kingdom Animalia to deacylate L-Ala-tRNA^{Thr}, which is a product of tRNA mis-selection by eukaryotic AlaRS. Evolutionarily, the emergence of ATD is linked to the tRNA explosion, wherein tRNA isodecoders (tRNA genes with sequence variation but having identical anticodon) containing G4●U69 are mischarged by eukaryotic AlaRS. ATD is the only known editing factor which is involved in proofreading tRNA mis-selection. To further understand the physiological significance of DTD and ATD, we are currently generating DTD knockout in fruit fly (*Drosophila melanogaster*), DTD and ATD knockouts in zebrafish (*Danio rerio*) and in mouse (*Mus musculus*). We are also exploring various aspects of other DTDs, namely DTD2 (present in archaea and plants) and DTD3 (found in cyanobacteria).

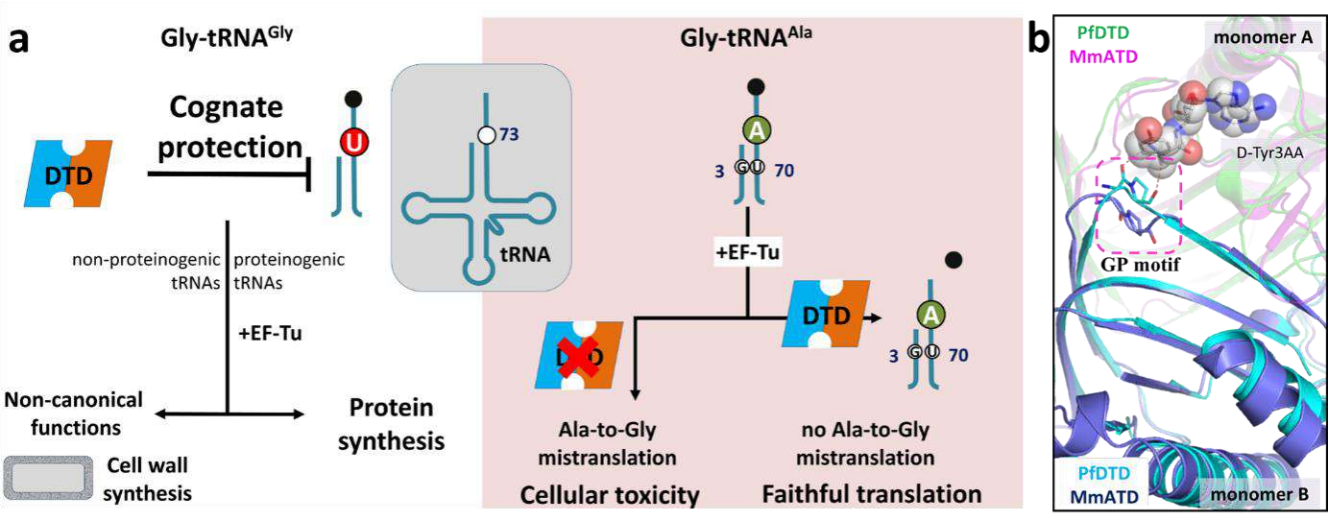


Fig. 1: a) Model depicting the role of N73 (discriminator) base of tRNA for bacterial DTD's action. U73 as the discriminator base of $tRNA^{Gly}$ avoids mis-editing of Gly-$tRNA^{Gly}$ (proteinogenic and non-proteinogenic), while efficiently edits Gly-$tRNA^{Ala}$ due to the presence of A73 and G3●U70 (Adapted from Kuncha et al., Elife 2018) **b)** Structural superimposition of *Mus musculus* ATD (MmATD) (PDB id: 5XAQ) on *Plasmodium falciparum* DTD (PfDTD) (PDB id: 4NBI) highlighting (pink box) the cross-subunit GP motif which is Gly-*trans*Pro in ATD while Gly-*cis*Pro in DTD.

The other major interest is to understand the structure, function, and mechanism of fatty acyl-AMP ligase (FAALs), an enzyme involved in the fatty acid activation for complex lipid and secondary metabolite biosynthesis. FAALs are variants of common ANL superfamily enzymes that are incapable of transferring the acyl-AMP intermediate to coenzyme-A (CoASH), instead, transfer it to *holo*-acyl carrier protein (ACP). The structural and mechanistic details of CoASH rejection and its fidelity to ACP are poorly understood and the current efforts are focused on unravelling such intricate mechanisms. Understanding the details of such a discriminatory mechanism will help in understanding the FAALs' functional importance in processes such as biosynthesis of virulent lipids and other bioactive molecules. We have also identified orthologs of FAALs in eukaryotic systems and it remains to be seen how and for what these unique mechanistic variants have been recruited in higher systems. Currently, we have generated the loss of function mutants of these genes in multiple model systems such as Yeast, Drosophila and Mouse to understand the basic function of these proteins. We are extensively utilizing the bacterial, yeast and insect cell line for expression of the eukaryotic proteins for structural and biochemical studies.

In collaboration with Dr. Ramesh Sonti's group, we are trying to understand the structure-function relationship of virulence factors of *Xanthomonas oryzae* pv. *oryzae* (Xoo), a plant pathogen causing bacterial blight of rice. The pathogen secretes a battery of plant cell wall-degrading enzymes by Type II secretion system. Our laboratory has solved the structure of catalytic domain of one of the cell wall degrading enzymes Cellobiosidase (CbsA). CbsA (catalytic domain) belongs to the Glycosyl hydrolase 6 (GH-6) family. Surprisingly, the canonical catalytic residues (D180 and D131/S137) mutation does not abrogate the biochemical activity of CbsA. Currently, we are trying to block the substrate-binding tunnel of CbsA by point mutations into bulky residues, to get a biochemically inactive enzyme. This inactive enzyme will be further assessed for its ability to induce immune response in rice. The C-terminal domain of CbsA is a Fibronectin type III domain (FN3). Recently, we have shown that in the presence of FN3 domain, CbsA catalytic domain does not act upon carboxymethylcellulose, a substrate on which the catalytic domain is otherwise active. Currently, we are trying to understand the molecular mechanism of this

inhibition using SAXS. With Yogendra Sharma's group, we have continued to explore various themes of calcium binding in microbial βγ-crystallins. DdcaD1 (*Dictyostelium discoideum* Ca^{2+}-dependent cell adhesion molecule-1 and APP (abundant perithecial protein) in *Neurospora crassa* are homologous proteins belonging to this class of βγ-crystallins. Here, we report how an interface in APP alters the calcium binding site. APP is a two domain protein, one domain is βγ- crystallin while other domain is immunoglobulin like (Ig-like). N-terminal domain (NTD) of APP is βγ- crystallin and is able to bind Ca^{2+} but the full length APP is incapable of binding calcium ion (Figure2.). Our work provides mechanistic insights into how protein interface makes the calcium binding site inoperative in APP. This point is further proven by disruption of interface that restores calcium binding.

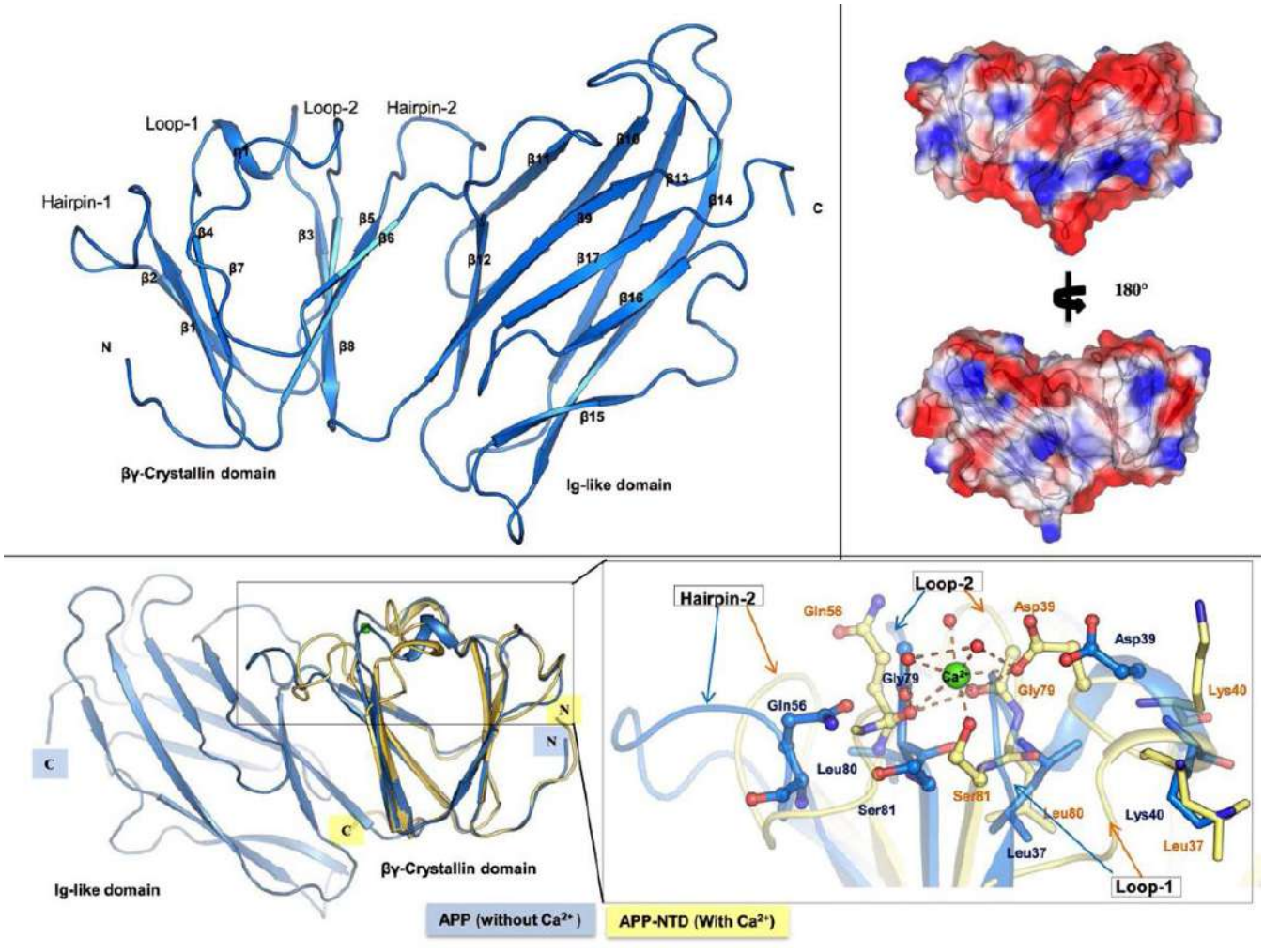


Fig. 2: Crystal structure of APP. A. Cartoon representation of crystal structure of APP from *Neurospora crassa*. B. Electronegative surface representation of APP in two orientations. C. Overlap of the crystal structures of APP (blue) and APP-NTD (yellow). Comparison of the Ca2+-binding site in APP (blue) and APP-NTD (yellow). From the structures, it is evident that the Ca2+-binding site seen in APP-NTD is completely remodeled into a cryptic site in the two domain parent APP. [Colour figure can be viewed at wileyonlinelibrary.com]

YOGENDRA SHARMA

Calcium Signaling via Calcium-binding Proteins

From left to right: P. Sai Uday Kiran, Radhika Khandelwal, Pranjal, Amrutha H C, Yogendra Sharma, Syed Sayeed Abdul, Manisha, Anand Kumar Sharma, R. Phanindra, Aditya Jamkhindikar, Asmita Dhansing Pawar, Bhim Bahadur, Venu Sankeshi

RESEARCH INTERESTS:

- βγ-Crystallin superfold and Ca^{2+} binding
- Bacterial immunoglobulin-like fold and Ca^{2+} binding
- Neuronal calcium sensors and pathophysiology

"Our group explores the properties of Ca^{2+}-binding proteins from all domains of life. In the bacterial systems, we were instrumental in the discovery and establishment of a novel Ca^{2+}-binding protein superfamily, i.e., βγ-Crystallins. Currently we are deciphering the anti-diabetic efficacy of a β-cell enriched Ca^{2+} sensor protein, Secretagogin"

Selected recent publications

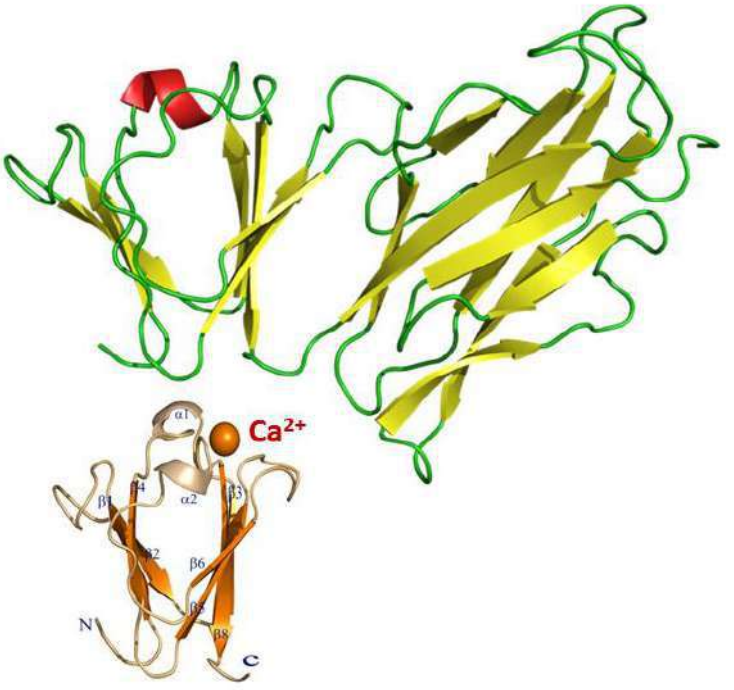
- Srivastava SS, Mishra A, Krishnan B, Sharma Y (2014). Ca^{2+}-binding motif of βγ-crystallins. *Journal of Biological Chemistry* 289: 10958-10966.
- Khandelwal R, Sharma AK, Chadalawada S, Sharma Y (2017). Secretagogin is a redox-responsive Ca^{2+} Sensor. *Biochemistry* 56: 411-420.
- Srivastava SS, Jamkhindikar AA, Raman R, Jobby MK, Chadalawada S, Sankaranarayanan R, Sharma Y (2017). A transition metal-binding, trimeric βγ-Crystallin from methane-producing thermophilic archaea, *Methanosaetath ermophila*. *Biochemistry* 56: 1299-1310.
- Swaroop Srivastava S, Raman R, Kiran U, Garg R, Chadalawada S, Pawar AD, Sharma Y (2018). Interface interactions between βγ-crystallin domain and Ig-like domain render Ca^{2+}-binding site inoperative in abundant perithecial protein of Neurosporacrassa. *Molecular Microbiology* 110: 955-972.
- Sharma AK, Khandelwal R, Sharma Y (2019). Veiled Potential of Secretagogin in Diabetes: Correlation or Coincidence? *Trends in Endocrinology & Metabolism* 30: 234-243.

We explore the properties of Ca^{2+}- binding proteins from all domains of life. In bacterial systems, we were instrumental in the discovery and establishment of a novel Ca^{2+}-binding protein superfamily, i.e., βγ-crystallins. Currently, we are deciphering the anti-diabetic efficacy of a β-cell enriched Ca^{2+} sensor protein, Secretagogin.

EF-hand motif containing proteins are the best-studied ones with respect to cation binding. Nevertheless, there are other motifs for Ca^{2+} binding, present in a wide variety of proteins. One such motif that we have identified is present in proteins belonging to the βγ-crystallin superfamily. We have classified βγ-crystallins as a separate class of Ca^{2+}-binding proteins, which constitute an expanding structural superfamily containing diverse members from various organisms. Despite being prevalent, in archaea, βγ-crystallins are selectively present only in three methanogens, i.e., Methanosarcina and Methanosaeta. M-Crystallin from *Methanosarcina acetivorans* has been reported by us as a Ca^{2+}-binding βγ-crystallin. 'Methallin' from *Methanosaeta thermophila*, the largest producer of methane, does not have the Ca^{2+}-binding motif. In collaboration with Dr. R Sankaranarayanan's group, we have demonstrated that instead of Ca^{2+}, Methallin binds a transition metal ion and forms a unique, ligand-dependent trimer. Engineering similar site in another, homologous βγ-crystallin led us to resolve that Methallin is a naturally designed trimer for high-affinity transition metal binding. While many βγ-crystallins are shown to bind Ca^{2+}, and form homodimers and oligomers, a transition metal-binding, trimeric βγ-crystallin is a new paradigm.

In another program under the broad interest of regulation of Ca^{2+} functions in eukaryotes, we have been characterising the selective calcium-sensing proteins, namely neuronal calcium sensor-1 (NCS-1), caldendrin, and secretagogin (SCGN). The transition of a calcium sensor from the apo (Ca^{2+}-free) to the holo (Ca^{2+}-saturated) state is a key event in calcium signalling which can be performed by sequential or simultaneous binding. The structural underpinnings for simultaneous vs. sequential Ca^{2+} filling are not yet known, but since many Calcium-binding proteins (CaBPs) supposedly follow a sequential mode of Ca^{2+} filling, it is likely that a CaBP will function *in vivo* at Ca^{2+} levels insufficient to saturate all binding sites. This is exactly what we have described recently that this transition may indeed be routed *via* stable transitory conformations and local reversible structural fluctuations in EF-hand motifs during hierarchical filling of Ca^{2+} in Caldendrin.

On our project of understanding the functions of Secretagogin (SCGN), which is a β-cell enriched, moderate affinity Ca^{2+} sensor; we have been exploring the inherent biochemical properties of SCGN and its implication in physiology. We are also interested in studying how pathological (or of unknown consequences) point mutations and SNPsaffect SCGN function. SCGN has also been implicated in the release of two critical hormones: insulin and corticotropin-releasing hormone. We have shown that SCGN displays redox-sensitiveoligomerisation and a higher affinity for Ca^{2+} in reducing milieu and gains stabilization. The redox-responsive nature of SCGN was supported by its response to the DTT-induced stress in MIN6 cells. Our article of redox-sensitive dimerization finds significance in the recent report implicating SCGN dimerization in insulin secretion.



APP, a two domain protein of βγ-crystallin and Ig-like domain does not bind Ca^{2+} despite the presence of a canonical Ca^{2+} binding site due to interface interference (upper). N-terminal βγ-crystallin domain binds Ca^{2+} (lower).

IMRAN SIDDIQI

Plant Reproductive Biology

From left to right: (First row) Komal Awallellu, Sai Kiran, Keith Frank Max, Bhaskar & Jayesh Davda
(Second row) Prashanthi, Mamta, Vishaka Bharadwaj, Aparna Singh, Veeraputhiran Subbiah & Imran Siddiqi
(Third row) Kaladhar Bethoju, Aswan Nalli, Sivakumar Prakash, Survi Mahesh, AV P Saradhi & Avinash Kumar Singh

RESEARCH INTERESTS:

- Plant Meiosis and Gametogenesis
- Apomixis
- Seed Development
- Epigenetics
- Plant Functional Genomics

"Analysis of meiosis and gametogenesis in plants offers opportunities for addressing fundamental questions relating to cell specification and in applying this information towards increasing food production through improved methods for plant breeding."

Selected recent publications

- Andreuzza S, Nishal B, Singh A, Siddiqi I (2015). The Chromatin Protein DUET/MMD1 Controls Expression of the Meiotic Gene TDM1 During Male Meiosis in Arabidopsis. *PLoS Genetics* 11: e1005396.

- Qin Y, Zhao L, Skaggs M, Andreuzza S, Tsukamoto T, Panoli A, Wallace KN, Smith S, Siddiqi I, Yang Z, Yadegari R, Palanivelu R (2014). ACTIN-RELATED PROTEIN6 regulates female meiosis by modulating meiotic gene expression in Arabidopsis. *Plant Cell* 26: 1612-1628.

- Marimuthu MPA, Jolivet S, Ravi M, Pereira L, Davda JN, Cromer L, Wang L, Nogue F, Chan SWL, Siddiqi I, Mercier R (2011). Synthetic clonal reproduction through seed. *Science* 331: 876.

Our research group works on meiosis and germ cell formation in plants. The work is aimed at understanding the control of meiosis and meiotic chromosome organization in plants, and in applying information obtained from a molecular genetic analysis of meiosis and gametogenesis towards developing new methods in plant breeding. These methods would involve fixation of hybrid vigour and accelerating plant breedingto meet the challenges facing agriculture with respect to increasing food production for a growing world population. Our group has identified and analyzed several genes that control core aspects of meiotic chromosome organization. Some of these genes are unique to plants whereas others show conservation to meiotic genes in other eukaryotes. The effort in the lab is towards developing an integrated understanding of the genetic and epigenetic controls regulating plant meiosis.

The plant life cycle consists of two distinct generations: a diploid sporophyte and a haploid gametophyte. As in other eukaryotes, meiosis is a key phase in the pathway of reproductive development. In plants meiosis also acts as a transition between the two generations. The analysis of plant meiosis is therefore of central importance in understanding early stages of plant reproductive development and is also of considerable practical significance with respect to the potential for manipulating meiosis and gametogenesis to advantage in plant breeding, for example in apomixis, Apomixis is the formation of asexual seeds in plants, and leads to populations that are genetically uniform.

Transfer of apomixis to crop plants holds great promise in plant breeding for fixation of heterozygosity and hybrid vigour as it would allow propagation of hybrids over successive generations and also accelerate breeding. Apomixis involves production of unreduced (diploid) female gametes that retain the genotype of the parent plant (apomeiosis), followed by parthenogenetic development of the egg cell into an embryo, and functional endosperm formation. Apomixis is found naturally in more than 400 species of flowering plants and can occur by distinct developmental routes. It has been suggested that this phenomenon results from deregulated expression of the sexual programme, however the molecular mechanisms that control apomixis are unknown. One hypothesis is that genes controlling apomixis may be variant alleles of genes that act during normal sexual development. Such genes may be revealed by analysis of model sexual plants. Our group has previously described a proof of principle demonstration for being able to generate clonal seeds in Arabidopsis by manipulating 2-4 genes involved in chromosome organization and segregation in plant meiosis. We are currently carrying out screens to identify additional genes that can be used to manipulate plant meiosis and initiation of seed development

Our recent studies on a newly identified gene *SHUKR* (*SKR*) reveal it to be expressed specifically in male meiosis and required for early stages of development of microspores into pollen.We have shown that SKR protein associates with chromatin and regulates the transition from the diploid sporophyte to the haploid gametophyte through control of protein homeostasis during pollen development. We have performed a genetic screen for suppressors of *skr* and have identified several suppressor mutants (*ssk*) which restore male fertility and seed set in a *skr* mutant background. Characterization of *ssk*mutants is in progress. Analysis of *SKR* and SSK genes is expected to provide novel insights into chromatin based mechanisms regulating the sporophyte to gametophyte transition.

Germ cell development involves integration of sex-specific developmental programmes with a common meiotic programme. In higher plants the fate of meiotic products are very different between male and female. In male meiosis all four spores formed after meiosis develop into pollen following

two rounds of post-meiotic division whereas in female meiosis three of the four spores degenerate and only one goes on to form a female gametophyte following three rounds of post-meiotic division. The *CDM1* gene encodes an RNA binding protein required for completion of male meiosis. We are studying the role of the *CDM1* gene in posttranscriptional control of male meiosis and identification of its molecular targets. We have recently screened for andidentified a suppressor of *CDM1* whose characterization should provide insights into the mode of action of CDM1 and its target genes.

In order to understand how the cell cycle controls governing initiation of embryogenesis, we have examined the cell cycle stage of the egg and sperm cells in Arabidopsis using markers for DNA synthesis and S phase. Our results provide novel information on the timing of DNA synthesis in male and female gametes in relation to fertilization and initiation of embryogenesis.

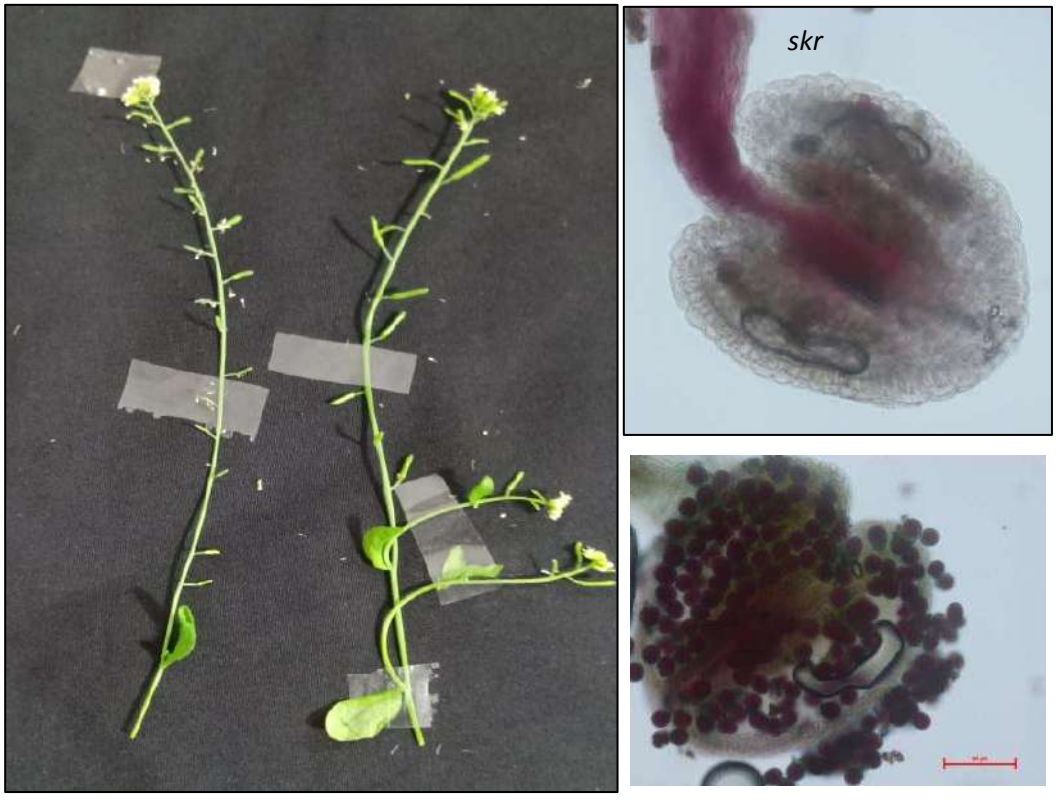


Fig. 1: Restoration of pollen viability in a *skr* suppressor mutant

PURAN SINGH SIJWALI

Roles of the Ubiquitin Proteasome System and Autophagy in Malaria Parasite Biology and Pathogenesis

From Left to Right: Renu Sudhakar, Divya Das, Puran Singh Sijwali, Manish Bhattacharjee, Deepak Kumar, Zeba Rizvi, Akshay, T. Nandita, Pushpita Saha, M. Angel Nivya, Savita, Navin Adhikari

RESEARCH INTERESTS:

- Ubiquitin proteasome system
- Autophagy
- Antimalarial targets
- Neddylation
- Cullin-ring-ligases
- Proteases
- Malaria vaccine

"We have shown that the malaria parasite autophagy protein Atg18 is associated with the parasite lysosome and is indispensable for parasite development. We have also established that Lyse-reseal erythrocytes for transfection (LyRET) of P. falciparum is a new cost-effective alternative to the existing electroporation-dependent transfection methods, particularly in resource-limited settings

Selected recent publications

- Gokulapriya Govindarajalu, Zeba Rizvi, Deepak Kumar and Puran Singh Sijwali. Lyse-Reseal Erythrocytes for Transfection of *Plasmodium falciparum. Manuscript under Revision.*

- Divya N. Nair, Rajesh Prasad, Neha Singhal, Manish Bhattacharjee, Renu Sudhakar, Pushpa Singh, Subramonian Thanumalayan, Uday Kiran, Yogendra Sharma, Puran Singh Sijwali (2018). A conserved human DJ1-subfamily motif (DJSM) is critical for anti-oxidative and deglycase activities of *Plasmodium falciparum* DJ1. *Molecular & Biochemical Parasitology* 222: 70–80.

We are studying the proteolytic systems of the malaria pathogen *Plasmodium* to determine their roles in parasite biology and disease pathogenesis. The multi-stage development of malaria parasites in different intracellular and extracellular host environments has been a major challenge to design/develop effective therapies for disease control/elimination. A major research interest of the lab is to identify proteins that have essential functions during parasite development and are parasite-specific or significantly different from the host homologs, so that they can be targeted for development of new drugs to control malaria.We are focusing on autophagy and the ubiquitin proteasome system (UPS), which have been shown to have crucial roles both in cellular homeostasis and regulatory processes.Inhibitors of these two machineries have been shown to kill malaria parasites, supporting their essential roles in parasite development and potential as drug targets.Autophagy and UPS involve several proteins with enzymatic activities, thereby offer multiple targets to attack the parasite.

Autophagy delivers a variety of cellular contents, including organelles, to the lysosome for degradation. It is also involved in selective transport of proteins and lipid catabolism. Since malaria parasites acquire and degrade several self and host cellular contents during their multi-stage development, we are characterizing various autophagy proteins to determine their functions during parasite development. The work from our and other laboratories previously showed a limited autophagy repertoire in malaria parasites. To investigate how Atg8-associated structures are formed, we are studying Atg18 and other proteins that are likely to have roles in generation of phagophore assembly sites (PAS) and formation of the phagophore, a cup-like Atg8 labelled structure. Localization and inhibition experiments indicated exclusive localization of Atg18 to food vacuoles, which are parasite's lysosomes wherein bulk degradation of haemoglobin occurs. This localization appears to be mediated by*binding of Atg18 with* p*hosphatidylinositol*3-phosphate on the food vacuole membrane. Some anti malarials showed a noticeable effect on Atg18 localization and expression, suggesting that Atg18 is another target by which these antimalarials kill the malaria parasite.Atg18 is essential for parasite development, asAtg18 gene could not be knocked-out and knock-down of Atg18 protein significantly reduced parasite growth. Studies are underway to dissect the autophagy repertoire and determine its roles in parasite development.

Along with studying the autophagy and UPS, we are developing simple and efficient transfection methods for genetic manipulation of *Plasmodium falciparum* to identify, characterize and validate the genes with therapeutic potential and better understand parasite biology. Among the available transfection techniques for *P. falciparum*, electroporation-based methods, particularly electroporation of ring-infected RBCs is routinely used. Nonetheless, transfection of *P. falciparum* remains a laborious and resource-intensive procedure. Here, we report a simple and economic transfection method for *P. falciparum*, which is termed as the lyse-reseal erythrocytes for transfection (LyRET). It involves lysis of erythrocytes with a hypotonic RBC lysis buffer containing the desired plasmid DNA, followed by resealing by adding a high salt buffer. These DNA-encapsulated lyse-reseal erythrocytes were infected with *P. falciparum* trophozoite/schizont stages and subjected to selection for the plasmid-encoded drug resistance. In parallel, transfections were also done by electroporation-based methods involving electroporation of DNA into uninfected RBCs (Epreloading) and ring stage-infected RBCs (EiRBCs). The LyRET method successfully transfected 3D7 and D10 strains with different plasmids in 47 of the 49 attempts, with success rate similar to transfection by electroporation of DNA into infected RBCs. In all the experiments, transfected expressed GFP (Fig. 1). The cost effectiveness and comparable efficiency of LyRET method makes it an alternative to the existing transfection methods for *P. falciparum*, particularly in resource-limited settings.

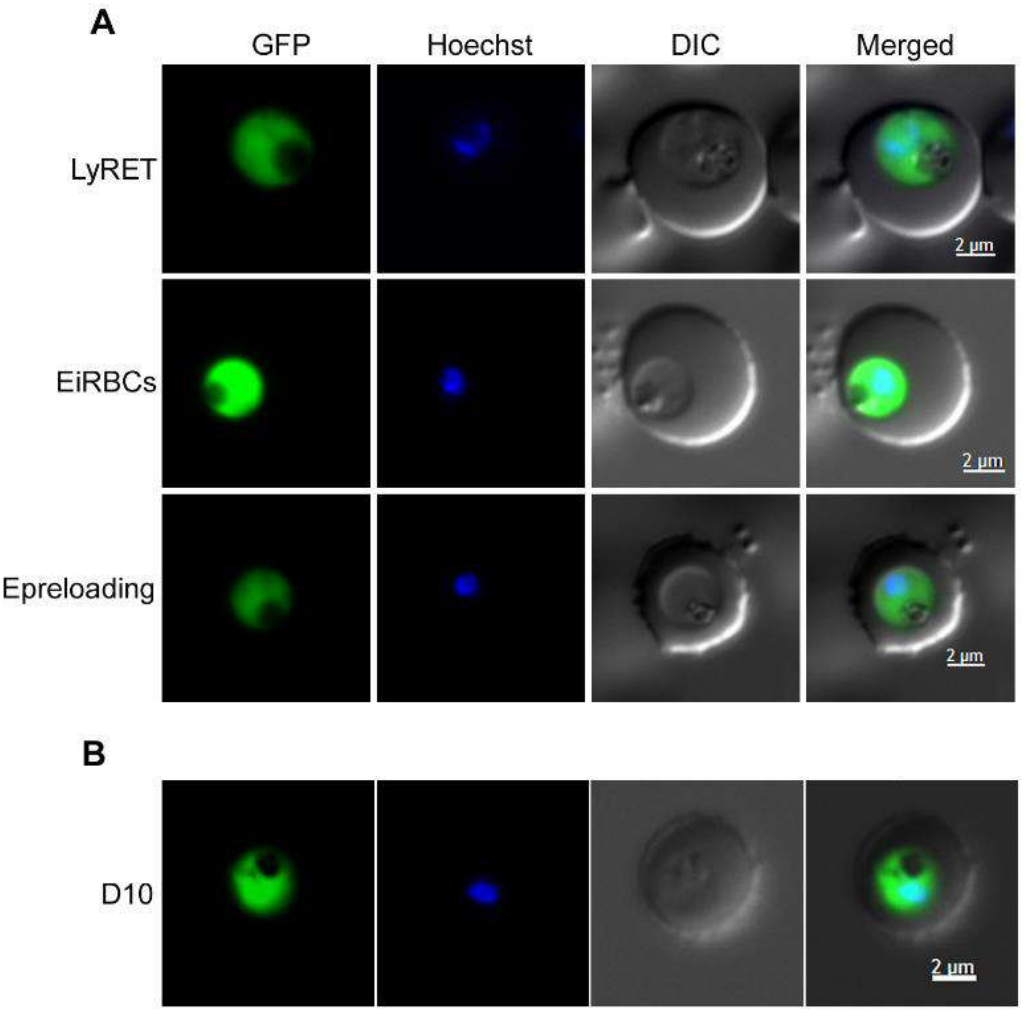


Fig. 1: GFP expression in transfected parasites. A.*P. falciparum* 3D7 strain was transfected with pPfCENv3 using LyRET, EiRBCs and Epreloading methods. B.*P. falciparum* D10 strain was transfected with pPfCENv3 using the LyRET method (D10). Drug resistant parasites were assessed for GFP fluorescence using live cell microscopy. Shown are the representative images for different methods. The panels in A and B are for GFP fluorescence in parasites (GFP), nuclear stain (Hoechst), bright field (DIC) and the overlap of all images (Merged). The presence of GFP indicates successful transfection.

SHASHI SINGH

Adult Stem Cells from various Sources and Tissue Engineering

Shashi Singh

RESEARCH INTERESTS:

- Tissue engineering using modified niches, natural scaffolds etc.
- Use of adult stem cells for lineage derivation by alteration of niche and small molecules etc.
- Creating human iPSC by viral, episomal and chemi-methods using fibroblasts and lymphocytes

"Matrix proteins like collagen Type I are used for creating cross-linked stable matrices for cell growth and differentiation of specific lineages. A series of constructs with different gums and protein ratios were screened and reveal that differential differentiation of cells depends on altered compositions"

Selected recent publications

- Rekulapally R, Murthy Chavali LN, Idris MM, Singh S (2019). Toxicity of TiO2, SiO2, ZnO, CuO, Au and Ag engineered nanoparticles on hatching and early nauplii of Artemia sp. *PeerJ* 6: e6138.
- Begum G, Reddy TN, Kumar KP, Dhevendar K, Singh S, Amarnath M, Misra S, Rangari VK, Rana RK (2016). An *in situ* Strategy to Encapsulate Antibiotics in a Bio-inspired CaCO3 Structure Enabling pH-Sensitive Drug Release Apt for Therapeutic and Imaging. *ACS Appl. Mater. Interfaces* 8: 22056-22063.

For regenerative medicine, stem cells can either be injected directly at the site of injury for injuries like spinal cord, bone repair, cardiac, etc., or tissue constructs in 3D can be created in culture conditions. The entire tissue can be reconstructed by tissue engineering such as cornea, liver, pancreas etc. We had been using MSCs obtained from tissues obtained as medical waste like placenta, Wharton's jelly and adipose aspirate. These cells were co-cultured with matrices composed of ECM components to make a cartilage construct. After obtaining an appropriate range of combinations, the experiments were repeated with iPSC obtained from human fibroblasts.

Human iPSC: While trying to screen small molecules obtained from plant products that could induce reprogramming in MSC for ES like phenotype; we created iPSC using lentiviral (clones K1, k2 and K3) and episomal induced iPSCs (clones M1, M2, L2, V1 and V3) with human fibroblasts. These cells have been characterized for expression of pluripotency markers by Immunofluorescence and PCR. These clones have been induced to make embryoid bodies that have been characterized for three germ layer induction. We presently have 8 clones of iPSC from the same set of fibroblast derived from anonymized fetal foreskin (18 weeks abortus). These cells are being used for differentiation into pancreatic, hepatic and brain organoids apart from cartilage constructs.

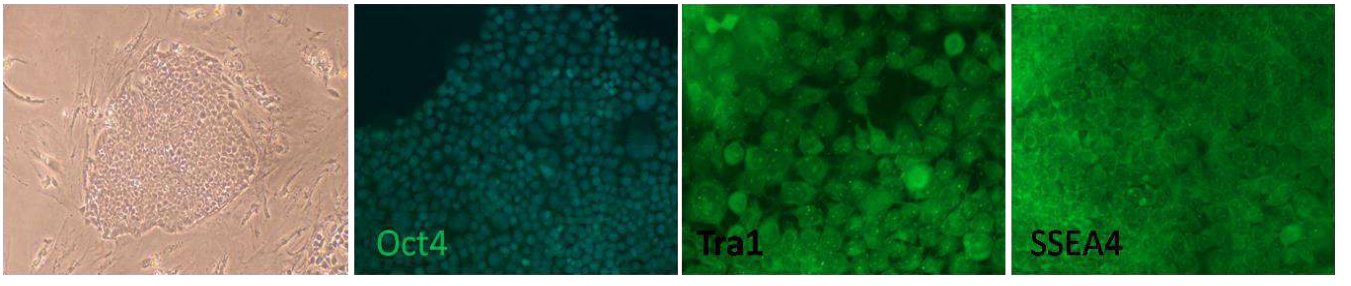


Fig. 1: Chracterization of an iPSC clone by immunofluorescence for pluripotency markers Oct4, SSEA4 and Tra1.

Differentiation of iPSC and their compatibility with Scaffolds

3D scaffolds of matrix proteins with plant based gums in form of hydrogels have been created for matrix driven differentiation of MSC. These constructs with predetermined ratio of glycans to protein (determined by experiments on MSC) have been used to induce iPSC into chondrocytes. iPSC were cultured entrapped in these hydrogels made up of collagen and glycans. The tissue construct blocks were examined by histochemistry, real time PCR and western blots. The glycan to protein ratio of the construct was determined after cell growth for a period of one month to see whether the cells are able to secrete fresh matrix. The cells were able to sustain and even multiply in the scaffolds. The cells appeared to have differentiated and some of the gels almost showed a histology pattern like chondrocytes (figure). Cells grown in scaffolds showed expression of chondrocytic markers like aggrecan and ovostatin, especially in gels which had a higher ratio of glycan to protein to begin with. Gels with 1% collagen with higher oxidation of glycan that results in good cross-linking of gels were found to be better in inducing chondrocytic behaviour in terms of expression of markers. PCR analysis of cells grown in gels showed that all the chondrocytic markers showed an increased expression whereas the expression of iPSC markers were reduced. Fresh matrix synthesis also could be seen in the gels showing chondrocytic differentiation. Western analysis of the constructs also showed protein expression of chondrocytic markers. The next step is to titrate the cells to matrix ratio for better outcomes. The animal model is being standardized for IVD injury and knee cartilage injury. These gels are in injectable form and set with 30 minutes time based on the glycan to collagen ratio.

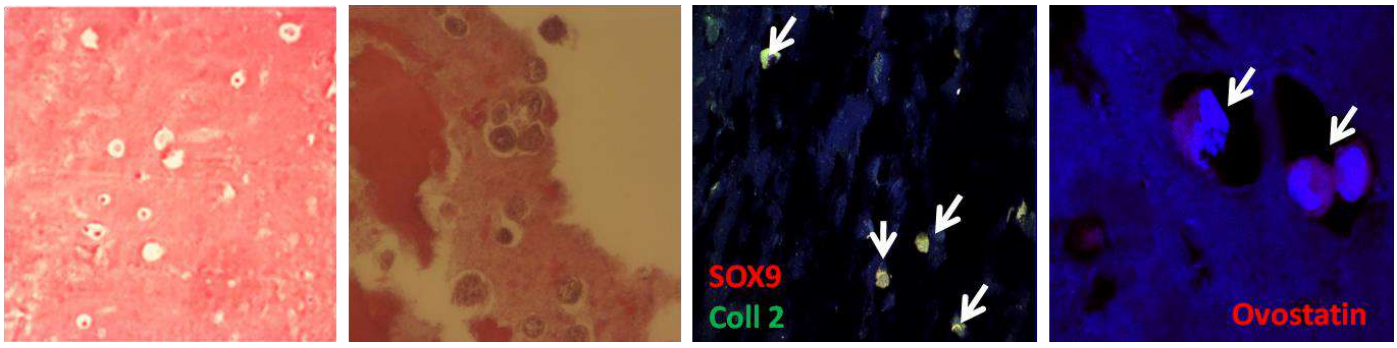


Fig. 2: Matrix induced differentiation of iPSC M1 into chondrocytes: Cells grown in hydrogel with 1% collagen cross-linked with gum arabica at 50% oxidation show chondrocyte like morphology and chondrocytic markers.

RAMESH V SONTI

Plant-Pathogen Interactions

From left to right: (First row) Gokulan CG, Ram Chandra Panigrahi, RajkanwarNathawat, Kamal Kumar M, Hitendra Kumar Patel, Rennya PR, Bipin Kumar
(Second row) Kranthi Brahma, KomalAwalellu, Namami Gaur, Sohini Deb, Palash Ghosh, Ramesh. V. Sonti
(Third row) Niranjani, Shailaja, PranaliVankore, Mani Deepika M, Raju Madanala
(Fourth row) Md. Jamaloddin, Vishnu NM, Donald James, Roshan MV

RESEARCH INTERESTS:

- Bacterial virulence functions
- Plant resistance responses
- Marker-assisted selection in plant breeding

"Induction and suppression of innate immunity in plants. Enhancing scope of marker assisted selection in plant breeding."

Selected recent publications

- Tayi L, Maku R, Patel HK, Sonti RV (2016). Action of multiple cell wall degrading enzymes is required for elicitation of innate immune responses during *Xanthomonas oryzae* pv. *oryzae* infection in rice. *Molecular Plant Microbe Interactions* 29: 599-608.

- Girija AM, Kinathi BK, Madhavi MB, Ramesh P, Vungarala S, Patel HK, Sonti RV (2017). Rice leaf transcriptional profiling suggests a functional interplay between *Xanthomonas oryzae* pv. *oryzae* lipopolysaccharide and extracellular polysaccharide in modulation of defence responses during infection. *Molecular Plant Microbe Interactions* 30: 16-27.

- Tayi L, Kumar S, Nathawat R, Haque AS, Maku RV, Patel HK, Sankaranarayanan R, Sonti RV (2018). A mutation in an exoglucanase of *Xanthomonas oryzae* pv. *oryzae*, which confers an endo mode of activity , affects bacterial virulence , but not the induction of immune responses, in rice. *Molecular Plant Pathology* 19: 1364-1376.

- Pillai SE, Kumar C, Patel HK, Sonti RV (2018). Overexpression of a cell wall damage induced transcription factor, *Os*WRKY42, leads to enhanced callose deposition and tolerance to salt stress but does not enhance tolerance to bacterial infection. *BMC Plant Biology* 18: 1-15.

- Deb S, Gupta MK, Patel HK, Sonti RV (2019). *Xanthomonas oryzae* pv. *oryzae* XopQ protein suppresses rice immune responses through interaction with two 14-3-3 proteins but its phospho-null mutant induces rice immune responses and interacts with another 14-3-3 protein. *Molecular Plant Pathology* doi: 10.1111/mpp.12807

Our group is studying the mechanisms by which microbes attack plants and plants defend themselves against microbial attack. The model system that we study is the interaction between the bacterial pathogen *Xanthomonas oryzae* pv. oryzae (*Xoo*) and it's host, the rice plant. A major focal theme of our research is to understand the mechanisms by which rice innate immune responses are induced and suppressed following bacterial infection. In previous work, we have shown that plant cell wall degrading enzymes, including a Lipase/Esterase (LipA), cellulase (endoglucanase; ClsA) and cellobiosidase (exoglucanase; CbsA) that are secreted by the *Xoo* pathogen induce plant defense responses. The *Xoo* secreted plant cell wall-degrading enzymes (CWDEs) are important virulence factors but the damage that they cause to plant cell walls, serves as a mark of infection that results in induction of defense responses. How does rice recognise this damage is still not clear. Our lab has identified a rice wall associated receptor kinase (WAK) that perceive cell wall damage cause by LipA and induce immunity. Expression of this gene is specifically because of presence of LipA in *Xoo*. Overexpression of this WAK in rice or Arabidopsis induces plant immune responses. This is a dual function enzyme as it possesses kinase and guanylate cyclase activity. Interestingly, in rice this WAK induce immunity by its kinase activity while in Arabidopsis it induces immunity by its guanylate cyclase activity.

In order to cause disease, *Xoo* suppresses these plant defense responses using a particular protein secretion system called the Type 3 secretion system (T3S) which secretes proteins directly into rice cells. A *T3S*$^{-}$ *Xoo* mutant is deficient in suppression of plant defense responses and is, in fact, an elicitor of plant defense responses because it continues to secrete plant cell wall-degrading enzymes. We have earlier shown that four of the *Xoo* T3S secreted proteins, are involved in suppression of cell wall damage-induced rice innate immunity. These proteins are called XopN (Xanthomonas Outer protein N), XopQ, XopX and XopZ. We have seen that this suppression is mediated by their interaction with the rice 14-3-3 proteins. The XopQ protein has two 14-3-3 binding motifs. Wild type XopQ protein interacts with two 14-3-3 proteins (Gf14f and Gf14g) of rice. Mutations in one of the 14-3-3 binding motifs of XopQ (S65A) affects the ability of XopQ to interact with Gf14f and Gf14g and also affects the ability of this protein to suppress rice immune responses. Further, overexpression of either Gf14f or Gf14g results in the induction of programmed cell death in rice roots and in enhanced tolerance to subsequent *Xoo* infection. These observations suggest that Gf14f and Gf14g are positive regulators of rice innate immune responses. We also postulate that interaction with XopQ affects the functioning of Gf14f and Gf14g in rice innate immunity and that this is how XopQ is able to suppress rice innate immunity. We also see that this mutant of XopQ, XopQ S65A induces the rice innate immunity by interaction with a third 14-3-3 protein, Gf14e, which is a negative regulator of immune responses. Taken together, our data suggests that XopQ may be functioning s a suppressor as well as inducer of the rice immune responses.

Lipopolysaccharide (LPS) is a structural component of the outer leaflet of the outer membrane of the bacteria. LPS is necessary for the function of various proteins and protect the bacteria from antimicrobial plant products. The *Xoo* LPS is a known elicitor of plant immune responses in rice. Exopolysaccharide (EPS) is a complex polymer secreted by *Xanthomonas* genus and known as xanthan gum.

The *Xoo* EPS is known to suppress the immune responses induced by LPS in rice. *Xoo* insertion mutants in EPS biosynthetic cluster and LPS outer antigen biosynthetic cluster are virulence deficit and display non-mucoid colony morphology due to the lack of EPS secretion. We have isolated *Xoo* colonies from stationary phase cultures, which exhibit non-mucoid morphology and are virulence deficient. These mutants are called as stationary phase variants (SPV). Our data shows that, SPVs arise due to insertion of endogenous insertion sequence (IS) elements in EPS or LPS O-antigen biosynthetic clusters or by strand slippage mutation (SSM) in *wxoA* gene of LPS O-antigen biosynthetic cluster. The SPVs revert to wild-type colony morphology and show true reversion, i.e. restoration of wild-type genomic locus. The results suggest that we are observing phase variation in *Xoo*.

In collaboration with ICAR-Indian Institute of Rice Research, Hyderabad, we have developed an ethyl methane sulfonate (EMS) induced mutant population in the background of an elite rice variety called Samba Mahsuri. A subset of this population was screened for various phenotypes including yield-related traits and biotic stress tolerance. Promising mutants were advanced to further generations through selfing to increase the homozygosity. With an aim to identify the genomic region(s) that contribute to the phenotype, we use a method called Bulked Segregant Analysis (BSA) in combination with Next-Generation Sequencing (NGS) technology and subsequent analysis. Our analysis revealed a possible candidate regions in the rice genome, mutations in which provide tolerance to a serious rice pest called Yellow Stem Borer (YSB). Subsequent to its validation, the identified mutations can be used as markers to impart the pest tolerance phenotype into other cultivars through breeding. This finding can help to prevent the damage caused by this pest in many rice-cultivated regions in the country. In addition, we are also working on mutant lines that show tolerance to a bacterial and a fungal disease of rice that cause huge damage to the crop across the country.

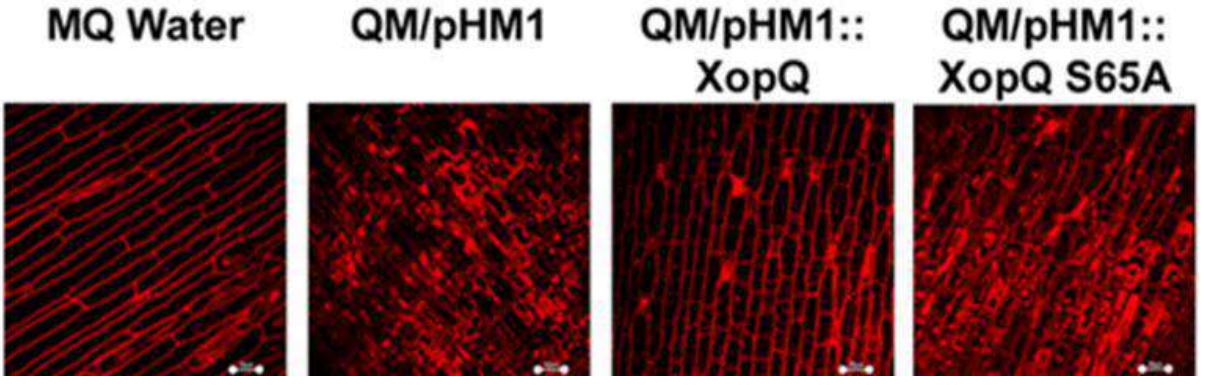


Fig. 1: The Serine-65 containing 14-3-3 protein binding motif of XopQ is essential for suppression of PCD in rice. Rice roots were treated with one of the following: MQ water, *xopQ xopN xopX xopZ* quadruple mutant (QM) containing any of the following constructs: *pHM1, pHM1::xopQ,* or *pHM1::xopQ S65A*. Treated roots were subsequently stained with propidium iodide (PI) and observed under confocal microscope using 63X oil immersion objectives. Bar, 20 µm. Internalization of PI is indicative of defence response-associated programmed cell death.

GHANSHYAM SWARUP

Molecular Mechanism of Neurodegeneration caused by Mutations in Optineurin

From left to right: Swetha Medchalmi, Ankita Singh, Ghanshyam Swarup, Zuberwasim Sayyad, Rajashree Ramaswamy, Shivranjani Moharir

RESEARCH INTERESTS:

- Molecular mechanisms of neurodegeneration caused by mutations in optineurin.
- Mechanisms of autophagy.
- Mechanisms of signal transduction by NLRC4, a mediator of innate immune response.

"The central theme of our group's research is to understand the function of the protein optineurin and to study the functional defects caused by disease-associated mutations in optineurin."

Selected recent publications

- Swarup G, Sayyad Z (2018). Altered functions and interactions of glaucoma-associated mutants of optineurin. *Frontiers in Immunology* 9: 1287.
- Bansal M, Moharir SC, Sailasree SP, Sirohi K, Sudhakar C, Sarathi DP, Lakshmi BJ, Buono M, Kumar S, Swarup G (2018). Optineurin promotes autophagosome formation by recruiting the autophagy-related Atg12-5-16L1 complex to phagophores containing the Wipi2 protein. *Journal of Biological Chemistry* 293: 132-147.
- Raghawan AK, Sripada A, Gopinath G, Pushpanjali P, Kumar Y, Radha V, Swarup G (2017). A Disease-associated Mutant of NLRC4 Shows Enhanced Interaction with SUG1 Leading to Constitutive FADD-dependent Caspase-8 Activation and Cell Death. *Journal of Biological Chemistry* 292: 1218-1230.
- Sirohi K, Chalasani ML, Sudhakar C, Kumari A, Radha V, Swarup G (2013). M98K-OPTN induces transferrin receptor degradation and RAB12-mediated autophagic death in retinal ganglion cells. *Autophagy* 94: 510-527.
- Vaibhava V, Nagabhushana A, Chalasani ML, Sudhakar C, Kumari A, Swarup G (2012). Optineurin mediates a negative regulation of Rab8 by the GTPase activating

protein TBC1D17. *Journal of Cell Science* 125: 5026-5039.

Molecular mechanisms of neurodegeneration caused by mutations in optineurin

Mutations in the coding region of the gene *OPTN*, which codes for the protein optineurin, cause glaucoma and ALS (amyotrophic lateral sclerosis) (Figure 1). Both of these are neurodegenerative diseases. In glaucoma, the loss of vision is irreversible, which occurs due to progressive degeneration of retinal ganglion cells (RGCs) in the optic nerve head. ALS is a fatal progressive disease, which involves degeneration of motor neurons of the primary cortex, brainstem and spinal cord. Optineurin is also seen in pathological structures present in Alzheimer's disease and Parkinson's disease. It is a multifunctional protein involved in signal transduction, membrane vesicle trafficking and autophagy. However, the precise role of optineurin in these functions and functional alterations caused by mutations in optineurin are not fully understood. A glaucoma-associated mutant of optineurin, E50K, induces cell death selectively in RGCs but some other mutants did not. This suggested that E50K mutant causes glaucoma possibly by directly inducing death of RGCs. E50K mutant induced death of RGCs is due to impaired vesicle trafficking as well as due to impaired autophagy, a quality control mechanism that is used by the cell to remove damaged proteins and organelles through lysosomal degradation. TBC1D17, a GTPase activating protein for Rab GTPases, plays a crucial role in E50K-induced impaired autophagy and impaired vesicle trafficking, and cell death.

M98K polymorphism is associated with glaucoma in certain ethnic groups. Like E50K mutant, M98K-OPTN induces cell death selectively in RGCs but not in other cells tested. But, unlike E50K-induced cell death, M98K-induced cell death is not inhibited by antioxidants or antiapoptotic protein Bcl2. Expression of M98K induces autophagy dependent cell death. Thus an appropriate level of autophagy is essential for survival of RGCs because altered autophagy, as seen with M98K-OPTN and E50K-OPTN, can lead to cell death.

To understand the function of optineurin in vivo, we have generated optineurin knockout mice by homologous recombination. These mice are born normally and do not show ALS phenotype or any other abnormality, suggesting, therefore, that the deficiency of optineurin is not sufficient to cause ALS in mice. These mice are being examined for various functional and cellular / molecular alterations in response to stress, such as ER stress.

We have re-characterized the photoreceptor cell line 661W because these cells could be differentiated into neuronal cells. Our results suggest that 661W cells are RGC precursor-like cells, which express several markers of RGCs and RGC precursor cells, and cone photoreceptor cells. These cells possibly represent a developmental stage just upstream of differentiated RGCs, as shown by expression of several molecular markers. This conclusion is supported by RGC-like property of selective induction of cell death by glaucoma-associated mutants of OPTN in these cells but not in other neuronal cells that were tested. Therefore, we suggest that these cells can be utilized for exploring the molecular mechanisms of RGC degeneration associated with glaucoma pathogenesis. We also observed that RGC-5 cells express the same RGC-specific and other molecular markers as seen in 661W cells.

Studies carried out on the pathogenesis of glaucoma using murine cell lines and animal models require to be validated in human cells. Human Primary Retinal Cells (hPRCs) in culture as a model for molecular studies and screening of potential therapeutic drugs have not been explored. Transition in conditions from in-vivo to ex-vivo can potentially alter the characteristics of retinal cells. In collaboration with Dr Inderjeet Kaur of L V Prasad Eye Institute, Hyderabad, we profiled adult hPRCs in culture for expression of various retinal cell specific markers and tested their sensitivity to disease associated mutants of OPTN. Our results suggest that hPRCs under appropriate cell culture condition appear to get dedifferentiated and show RGC-like properties.

These cells can possibly be used to explore the molecular mechanisms of glaucoma pathogenesis and for drug testing.

Mechanisms of autophagy

During autophagy, signaling events lead to formation of a cup-shaped structure called the phagophore that matures into the autophagosome, a double-membrane structure that plays a crucial role in recruiting the cargo and delivering it to lysosome for degradation. Fibroblasts from Optn knockout mouse showed a lower number of autophagosomes and autolysosomes during both basal and starvation-induced autophagy. However, the number of phagophores was not decreased in Optn-deficient cells. Phosphorylation of optineurin at S177 was required for autophagosome formation. Our results suggest that optineurin potentiates LC3-II production and maturation of phagophore into autophagosome, by facilitating the recruitment of Atg12-5-16L1 complex to Wipi2-positive phagophore. An ALS-associated mutant E478G-OPTN is defective in autophagosome formation. Various functions of optineurin in autophagy are summarised in Figure 2.

We have identified and characterized a splice variant of mouse optineurin which produces a truncated protein lacking N-terminal 157 amino acids (d157mOptn). This mRNA and protein are expressed in several tissues and cells. It is defective in cargo-selective and non-selective autophagy possibly due to loss of phosphorylation, impaired interaction with TBK1, and impaired recruitment to the phagophore. Our results provide an insight into the role of N-terminal domain of Optn in various autophagic functions.

Mechanisms of signal transduction by NLRC4, a mediator of innate immune response

Our innate immune system responds to invading microorganisms, which are recognized by cell surface (Toll-like receptors) or cytoplasmic receptors (Nod-like receptors, NLRs) to induce a protective response. NLRC4 (Ipaf), a NLR family member, activates caspase-1 in response to intracellular pathogens to induce production of proinflammatory cytokine interleukin-1 beta. We have shown that NLRC4 is a transcriptional target of p53 and is required for induction of cell death in response to chemotherapeutic drugs. The mechanisms, which are involved in the activation of NLRC4 in response to upstream signals such as those generated by pathogen infection or chemotherapeutic drugs are not fully understood. In collaboration with Dr. V. Radha, we identified a novel interaction between NLRC4 and the proteasome component Sug1, which enables ubiquitination of NLRC4 leading to FADD-dependent caspase-8 activation and cell death. Phosphorylation of NLRC4 at Ser533 plays a crucial role in caspase-8 activation and cell death.

Point mutations in NLRC4 cause autoinflammatory syndromes. Though all the mutations result in constitutive caspase-1 activation their phenotypic presentations are different, implying that these mutations cause different alterations in properties of NLRC4. We showed that the auto-inflammation associated H443P-NLRC4 mutant is altered in interaction with SUG1 and ubiquitinated proteins, triggering constitutive caspase-8 mediated cell death dependent on FADD, but independent of Ser533 phosphorylation. H443P-NLRC4 mutant causes familial cold autoinflammatory syndrome. Individuals carrying this mutation show autoinflammation, and symptoms get aggravated upon exposure to low temperature. H443P-NLRC4 mutant induced inflammasome formation and caspase-1 activation are enhanced upon exposure to low temperature, but the mechanisms of temperature sensing are not known. Currently we are investigating these mechanisms.

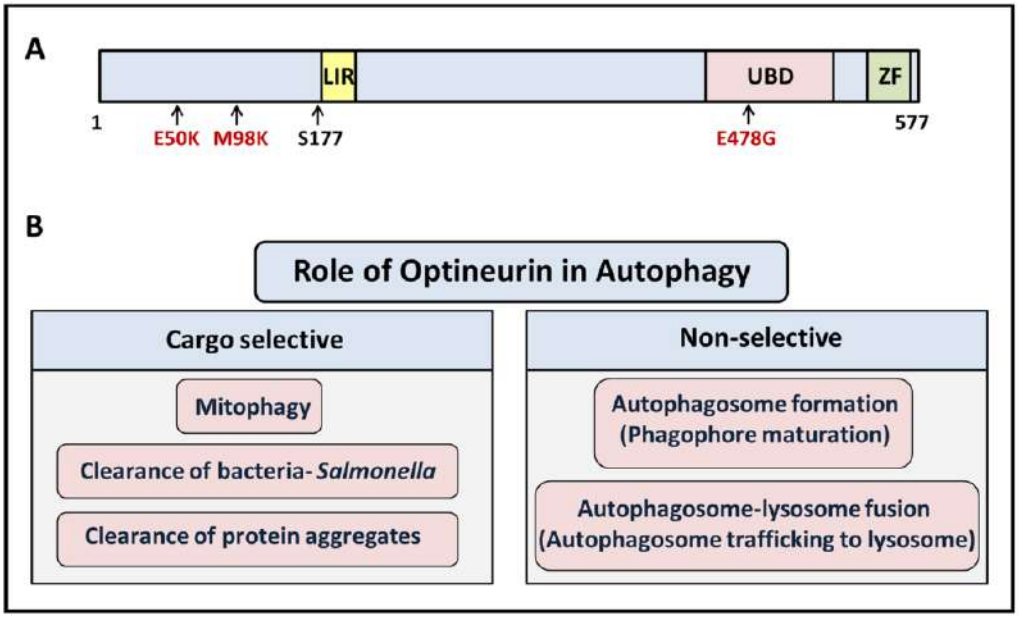


Fig. 1: (A) Schematic showing functional domains and some mutations in human optineurin. E50K and M98K mutations are associated with glaucoma, whereas E478G is associated with amyotrophic lateral sclerosis. LIR, LC3-interaction region; UBD, ubiquitin-binding domain; ZF, zinc finger. **(B)** Various functions of optineurin in autophagy.

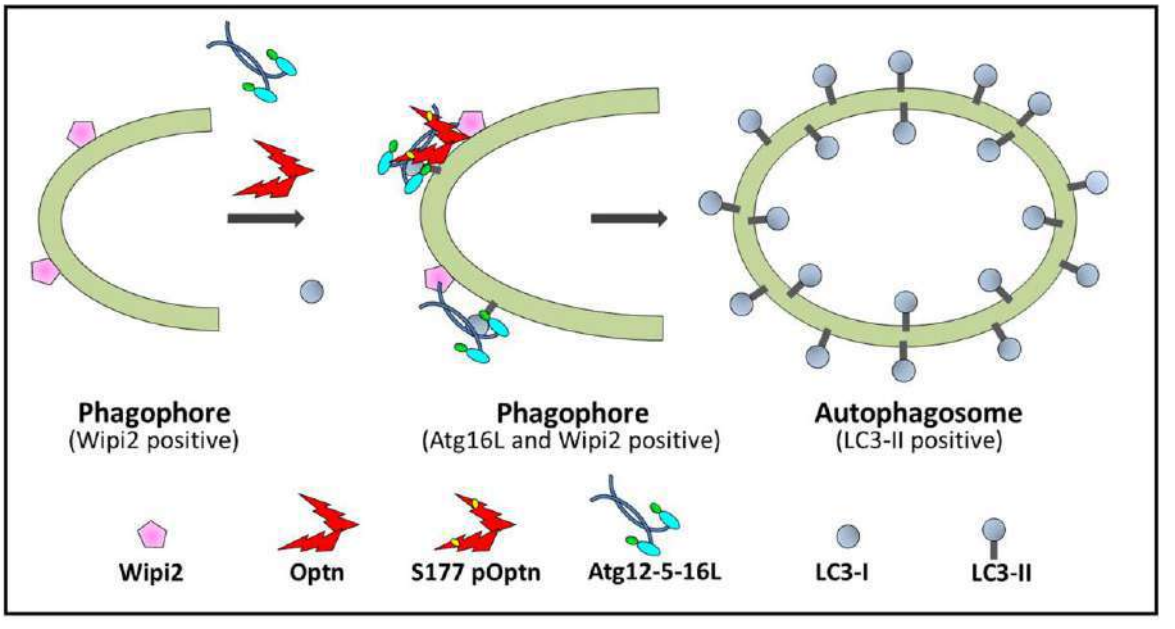


Fig. 2: Optineurin promotes autophagosome formation by facilitating the recruitment of the enzyme Atg12-5-16L1 complex to the Wipi2-positive phagophore. In Optn-positive (wild type) cells, recruitment of Atg12-5-16L1 complex to the phagophore is enhanced that results in higher production of LC3-II, which is required for expansion and closure of the phagophore to form autophagosome. Phosphorylation of optineurin at S177 is required for promoting autophagosome formation, and phospho-optineurin is seen on the phagophore.

RAGHUNAND R TIRUMALAI

Dissecting the Physiology and Pathogenic Mechanisms of *Mycobacterium tuberculosis*

From left to right: Amit Kumar, Juhi, Raghunand Tirumalai, Shilpi Dash, Ravi Prasad Mukku

RESEARCH INTERESTS:

- Host-pathogen interactions during *M.tb* infection
- Identification of mycobacterial virulence determinants
- Mechanisms of antibiotic resistance in mycobacteria

"Our research is focussed towards understanding the mechanisms of establishment and persistence of *M.tb* infection in its human host"

Selected recent publications

- Viswanathan G, Md. Jafurulla, Kumar GA, Raghunand T.R*, Chattopadhyay, A* (2018). Macrophage sphingolipids are essential for the entry of mycobacteria. *Chemistry and Physics of Lipids* 213: 25-31.
- Viswanathan G, Yadav S, Raghunand TR (2017). Identification of mycobacterial genes involved in β-lactam sensitivity - Implications in treatment of tuberculosis with β-lactam containing regimens. *Antimicrobial Agents and Chemotherapy* 61: e00425-17.
- Viswanathan G, Yadav S, Joshi SV, Raghunand TR (2017). Insights into the function of FhaA, a cell-division associated protein in mycobacteria. *FEMS Microbiology Letters* 364: fnw294.
- Yeruva VC, Savanagouder M, Khandelwal R, Kulkarni AM, Sharma Y, Raghunand TR (2016). The *Mycobacterium tuberculosis* desaturase DesA1 (Rv0824c) is a Ca^{2+} binding protein. *Biochemical and Biophysical Research Communications* 480: 29-35.

- Yeruva VC, Kulkarni AM, Khandelwal R, Sharma Y, Raghunand TR (2016). The PE_PGRS proteins of *Mycobacterium tuberculosis* are Ca^{2+} binding mediators of host-pathogen interaction. *Biochemistry(ACS)* 55: 4675-4687.

The extraordinary success of *Mycobacterium tuberculosis* (*M.tb*), the etiologic agent of human tuberculosis (TB), has been attributed to its ability to modulate host immune responses, facilitating its long term persistence. Control over the infection is complicated by the complexity of host-pathogen interactions where initial infection is followed by bacterial multiplication within mononuclear phagocytes, release of intracellular organisms, and dissemination. The identification and characterisation of bacillary factors involved in evasion, and their interplay with host defense components during infection is vital to understanding the pathogenic mechanisms of *M.tb*. We believe that understanding the basic biology of the pathogen and its interactions with the host is the best way forward towards the development of improved diagnostic reagents, vaccines and novel anti-TB drugs. Research in our laboratory is focussed towards characterising the events at the host-pathogen interface, the identification of bacillary virulence factors and identifying novel antibiotic resistance mechanisms.

Deciphering the functional role of unusual PE/PPE gene clusters in the pathogenesis of *M.tb*

The genome of *M.tb* encodes multiple immunomodulatory proteins, including several genes of multigenic PE_PPE (named after the conserved Proline-Glutamate and Proline-Proline-Glutamate residues at their N-termini) family, which comprise about 10% of its coding potential. The presence of these proteins in pathogenic mycobacteria strongly suggests that they play a role in disease pathogenesis. Several of the PE_PPE genes are organised in clusters that include *PE-PPE, PE-PE and PPE-PPE* genes arranged in tandem. While the functional properties of individual PE/PPE proteins have been examined, little information exists on the integrated functions of such gene clusters. To understand their role in *M.tb* physiology we have begun to characterise the *PPE50 (Rv3135)-PPE51 (Rv3136)*, and *PPE25 (Rv1787)-PE18 (Rv1788)-PPE26 (Rv1789)*gene clusters. The former is one of four *PPE-PPE* clusters in the *M.tb* genome and the latter, which is situated in the ESX-5 type-VII secretion system of *M.tb,* is the only cluster with this kind of organisation. Using RT-PCR we demonstratedthat these clustersare operonic, based on which we hypothesised that they encode interacting proteins, a common feature of operons. Using Mycobacterial Protein Fragment Complementation (M-PFC) we observed that the PPE50 - PPE51 and PPE25 - PPE26 protein pairs interact *in vivo*. This observation was validated by *in vitro* pull-downs using purified preparations of these proteins. We expressed these as c-myc fusions in the surrogate saprophytic host *Mycobacterium smegmatis* and observed that they localise to the cell surface, making this model competent to examine the roles of these proteins in host-pathogen interaction. CFU counts of THP-1 macrophages infected with recombinant *M. smegmatis*strains expressing *PPE50* and *PPE51* individually and in combination suggested that PPE50 and PPE51 play a role in intracellular bacillary survival (Fig. 1). In pull-down experiments, PPE50 was observed to interact with TLR1 (Fig. 2), consistent with its probable role in immune signaling. We are now performing cytokine profiling experiments to study the possible immunomodulatory functions of these proteins during infection.

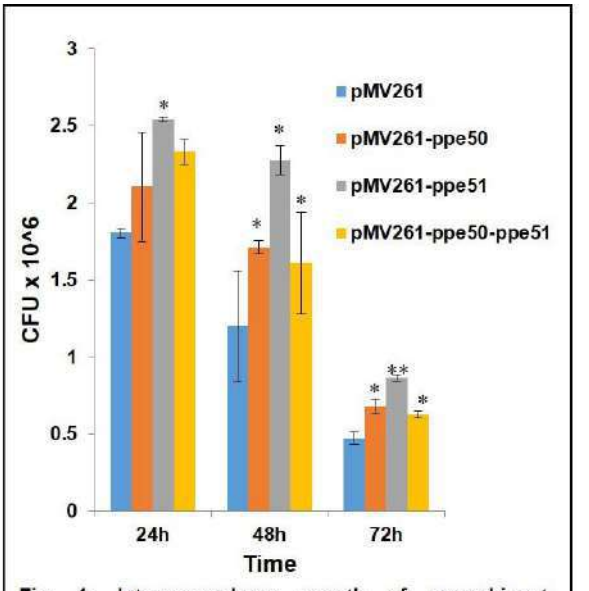


Fig. 1: Intramacrophage growth of recombinant *Mycobacterium smegmatis* strains. CFU counts of *M.smegmatis* expressing pMV261, PPE50, PPE51, and PPE50/PPE51, 24, 48 and 72 h post infection. * P<0.01, ** P<0.005

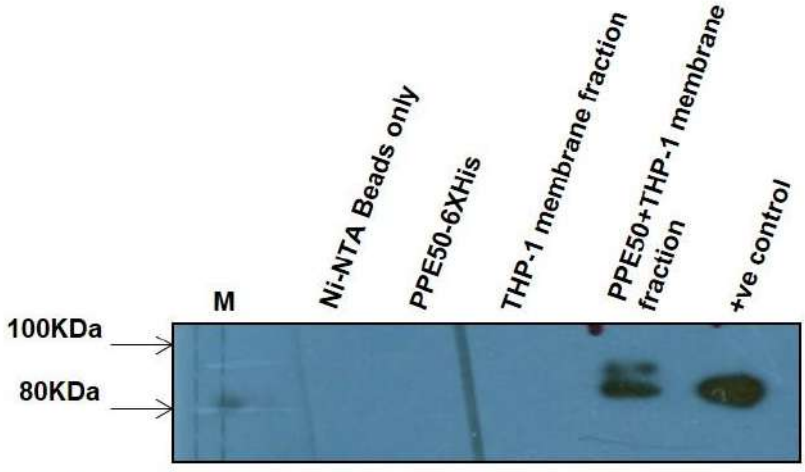


Fig. 2: Receptor interaction assay - Pull down performed by immobilizing PPE50-6XHis on Ni-NTA beads followed by Western blotting with an anti-TLR1 antibody

SHRISH TIWARI

Sequence Analysis of Biomolecules

From left to right: (Front Row) P. Ramesh, Pranali Vankore, Shrish Tiwari, Deepti Rao
(Back Row) Ram Chandra Panigrahi, Deepak Sharma, G. Hitesh, Satuluri Sri Harsha

RESEARCH INTERESTS:

- NGS sequence analysis, including *de novo* assembly and variant identification
- Discovering correlation between genotype and phenotype

"Our group is interested in the analysis of variants in the functional regions of the genome to unravel genotype to phenotype correlations. In particular, whole genome sequences of rice with agronomically favourable traits are being screened for loci which could be responsible for these traits."

Selected recent publications

- Muripiti V, Mujahid TY, Boddeda VHV, Tiwari S, Marepally SK, Patri SV, Gopal V (2018). Structure-activity relationship of serotonin derived tocopherol lipids. *International Journal of Pharmaceutics* 554: 134-148.
- Shrivastava S, Jafurulla Md, Tiwari S, Chattopadhyay A (2018). "Identification of Sphingolipid-binding Motif in G Protein-coupled Receptors" In: Chattopadhyay K., Basu S. (eds) *Biochemical and Biophysical Roles of Cell Surface Molecules. Advances in Experimental Medicine and Biology*, vol 1112. Springer, Singapore.
- Khurana R, Verma VK, Rawoof A, Tiwari S, Nair RA, Mahidhara G, Idris MM, Clarke AR, Kumar LD (2014). OncomiRdbB: a comprehensive database of microRNAs and their targets in breast cancer. *BMC Bioinformatics* 15: 15.
- Chattopadhyay A, Paila YD, Shrivastava S, Tiwari S, Singh P, Fantini J (2012). Sphingolipid-binding domain in

the serotonin$_{1A}$ receptor. *Advances in Experimental Medicine and Biology* 749: 279-293.

- Paila YD, Tiwari S, Sengupta D, Chattopadhyay A (2011). Molecular modelling of the human serotonin$_{1A}$ receptor: role of membrane cholesterol in ligand binding of the receptor. *Molecular Biosystems* 7: 224-234.

We have done some work in the area of prediction of non-coding RNAs and other regulatory motifs in the genome. At present this is restricted to a comparative genomics approach, but with the availability of genomes from closely related species, this approach is becoming increasingly powerful and accurate. Using a combination of experimental and computational approaches we have shown that a non-coding RNA expressed from the distal heterochromatin of the human Y chromosome contributes the 5'UTR of a testis-specific isoform of the autosomal gene CDC2L2. We have also uncovered a piRNA cluster on the mouse Y chromosome, which may be regulating autosomal genes.

Our search for SNPs that might be used as diagnostic markers resulted inaweb-based platform, MtSNPscore that helps to prioritise SNPs which may be involved in mitochondrial diseases. MtSNPscore computes a score for every SNP in the mitochondrial genes that has a significantly higher frequency in patients compared to normal individuals. It takes inputs from several analysis programs to assess the damaging effect of the SNP, the higher the score, the more damaging the SNP. The score of each SNP in a patient is used to assess their combined effect. It was applied to a dataset of 90 patients suffering from sporadic ataxia, where all nuclear factors were eliminated, and was shown to be highly discriminatory, with no false positives, *i.e.* no normal individual was predicted to have ataxia based on their score. It was further tested on 6 Japanese datasets, each consisting of 96 mitochondrial genomes of individuals with a particular phenotype. The 6 phenotypes were individuals with alzheimer's, parkinson's, diabetes with and without angiopathy, obesity and thin phenotype.In these studies we found that occasionally synonymous SNPs were also associated with a phenotype. This can happen when a rarely used codon is mutated to a more frequently used one, or vice versa. A possible explanation is that the rare codon introduces a translational pause, which allows the already formed protein chain to partially fold, thus avoiding aggregation. The translational machinery gets disrupted due to the mutation leading to misfolding of the protein. We are exploring this phenomenon, with an aim to predict which synonymous changes lead to altered protein function.

With the advent of next generation sequencing technology, genome sequencing has become routine. We have sequenced the elite rice variety known as Samba Mahsuri (SM) and its blight resistant variety, obtained through a marker assisted back-cross breeding program, named Improved Samba Mahsuri (ISM) and a sister line of ISM (SISM).We would like to identify the regions of the donor rice introgressed into SM in the back-cross breeding program, apart from the selected loci. A cluster of SNPs unique to ISM and SISM that are not present in SM are expected to lie in regions introgressed from the donor variety. At present, since we do not have the complete genome of SM, this involves aligning the reads from SM, ISM and SISM to the Japonica genome and then sifting out SNPs that are present in ISM and SISM and absent in SM. We are in the process of building the SM genome, so that it can act as the reference genome in our future analyses. We are also in the process of analysing EMS mutant lines of rice with agronomically useful traits, using an approach known as MutMap, with the hope of identifying the genes/loci responsible for the phenotype. In the MutMap approach stable EMS mutants, with a favourable agronomic trait, are crossed with wild-type parental line to get F1 generation. F1 is selfed to get F2 generation, which segregates for mutant and wild-type phenotype. The plants exhibiting the mutant phenotype are bulked and sequenced along with the parental plant. The aim is to identify a cluster of SNPs that is present in almost all the mutant plants. These loci are predicted to be responsible for the observed trait. The EMS mutant we are working with has the properties of

early flowering and high yield. We are at present focussing our attention on the early flowering phenotype.

We have also sequence a halophyte plant, *Salicornia brachiata*, that accumulates salt in its stems. This is a plant from which out sister lab CSIR-CSMCRI has been able to extract herbal salt, a low sodium salt from botanic origin. We have obtained the Ilumina reads for the complete genome. We also plan to sequence the genome using the PacBio approach, which will give us longer reads and help in a better assembly of the genome.

Our group has also done some molecular modelling and docking studies on the GPCR serotonin 1A receptor. In particular we showed that the receptor with bound cholesterol has a more compact structure. Several agonists and antagonists, including serotonin, were docked on the structure predicted through homology modelling. We see that the binding sites for antagonists were relatively shallow compared to those of the agonists. We also studied the evolutionary conservation of amino acid motifs involved in the binding of sphingolipids and cholesterol.

TUSHAR VAIDYA

Molecular Analysis of Host-Pathogen Interactions

From left to right: (Front Row) Satyjeet, Ram Prasad (Back Row) Tushar Vaidya, Pradyumna, Devi Prasad

RESEARCH INTERESTS:

- Virulence mechanisms in *Leishmania*
- Host immune responses to parasiticinfections
- Generation of immune memory

"Our research explores the interplay between a pathogen's efforts to establish infection in a host juxtaposed against the host's immune response to prevent infection"

Selected recent publications

- Rudra Chhajer, Anirban Bhattacharyya, Nicky Didwania, Md Shadab, Nirupam Das, Partha Palit, Tushar Vaidya, Nahid Ali (2016). *Leishmaniadonovani* Aurora kinase: a promising therapeutic target against visceral leishmaniasis. *Biochemica et Biophysica Acta* 1860: 1973–1988.

- Srijani Basu, Sheetal Kaw, Lucas D'Souza, Tushar Vaidya, Vineeta Bal, Satyajit Rath, Anna George (2016). Constitutive CD40-signaling calibrates differentiation outcomes in responding B cells via multiple molecular pathways. *Journal of Immunology* 197: 761-770.

- Mala Upadhyay, Krishna Priya G, Ramesh P, Madhavi MB, Satyajit Rath, Vineeta Bal, Anna George, Tushar Vaidya (2014). CD40 Signaling Drives B Lymphocytes into an Intermediate Memory-Like State, Poised Between Naïve and Plasma Cells. *Journal of Cellular Physiology* 229: 1387–1396.

- Santosh K Panda, Sunil Kumar, Nitin C Tupperwar, Tushar Vaidya, Anna George, SatyajitRath, Vineeta Bal and Balachandran Ravindran (2012). Chitohexaose

activates macrophages by alternate pathway through TLR4 and blocks Endotoxemia. *PLOS Pathogens* 8: e1002717.

- Vidhi Puri, Aneesh Goyal, Rajan Sankaranarayanan, Anton J Enright, Tushar Vaidya (2011). Evolutionary and functional insights into Leishmania META1: evidence for lateral gene transfer and a role for META1 in secretion. *BMC Evolutionary Biology* 11: 334.

Our laboratory is interested in understanding host pathogen interactions that influence the outcome of infections. Our model system is the protozoan pathogen, *Leishmania donovani*. *Leishmania* are transmitted from one human to another by an insect vector, the sand fly. In the insect, *Leishmania* exist as free-living, motile Promastigotes while within the mammalian host, *Leishmania* reside within macrophages as immotile Amastigotes. Our long-term objectives are studying molecules and mechanisms that confer any advantage to *Leishmania* during the various stages of its life cycle.

A *Leishmania* gene, META1, encodes a candidate virulence molecule. Virulent *Leishmania* cells have greater *META1* expression than in attenuated cells, over a wide range of conditions and environments. Using multiple criteria including sequence similarity, nucleotide composition, phylogenetic analysis, selection pressure on sequence etc., we established that *Leishmania META1* has been acquired through an ancient lateral gene transfer event between bacteria and a trypanosomatid ancestor. Superposition of META1 sequence on the solved structure of *Shigella*MxiM, has highlighted a putative hydrophobic cavity in META1. We mutagenized select hydrophobic residues in this cavity - this affected the secretion of the secreted acid phosphatase (SAP), indicating the involvement of META1in secretory processes in *Leishmania*. In ongoing experiments, we observed that overexpression of META1 causes a morphological reduction in size of *Leishmania*. In late stationary phase cells, greater than 90 % of META overexpressing Leishmania are one-fifth the size of the wild type cells (contrast to < 5% in wild type *Leishmania*). This phenotype is completely reversible; cells regain their size within 72 hrs, when they are fed with fresh media. The observed reduction in cell size is not associated with cell death nor induced merely by absence of nutrition or by the presence of spent media. Instead, parallel experiments suggest that META overexpression reduces the uptake and/or utilisation of nutrients. In both wildtype and META1 over expressing *Leishmania* there is an ~20 fold increase in META1 transcript at the time of the morphological reduction. However, upon refeeding the cells with fresh media, both the endogenous and ectopically expressed transcript levels reduce back to log phase levels, consistent with reversal in cell morphology. This also suggests a post-transcriptional control on META1 levels that limits the amount of META1 in a stage specific manner. A specific point mutation in the putative pocket of META1 protein already shown by us to associate with increased secretion, also diminishes the cells ability to undergo this transition. This, together with our observation of reduced nutrient uptake, underscores role for META1 in *Leishmania* interactions with its environment. Quite surprisingly, while overexpression of META1 derived either from *L. donovani* (LdMETA1) and *L. major* (LmjMETA1) can induce a reduction in cell size against *L. Donovani* background, both genes fail to do so in a *L. major* cell background, alluding to a possible role for META1 in tissue tropism.

In addition to this, we have identified a novel pair of paralogous genes in *Leishmania*, which we have named Differential Regulated Genes, DRG1 and DRG2. In all *Leishmania* species examined, *DRGs* occur as two paralogs (*DRG*1 and *DRG*2) on the same chromosome, each gene encoding virtually identical proteins except for the last six amino acid residues. The DRG proteins are predicted to have two transmembrane domains, with the C-terminal transmembrane domain being highly conserved in all related Trypanosomatids. Interestingly, despite coding for such closely related proteins, DRGs differ in stability, localization and function. We generated transgenic *Leishmania* ectopically singly expressing either a) DRG1 unique C-terminal domain or b) DRG2 unique C-terminal domain and c) DRG, just the

common DRG sequence, fused to the reporter protein, GFP. Confocal microscopy studies established that both the respective unique domains and the common domain are necessary for appropriate native localization of the DRG protein.

Our present interest in the DRG story is the differential regulation of DRG1 vs. DRG2 transcripts, in particular, the role of the evolutionarily conserved respective 3'UTRs. In *Leishmania*, gene regulation is thought to occur primarily at a post-transcriptional level. We initiated this effort by using bioinformatics approaches to predict likely cis-acting RNA elements (Pyrimidine rich tracts + COSMOS, a Statistical approach for Conserved Motif Search across species) in DRG1 vs. DRG2 mRNAs as well as the likely RNA binding proteins (RPI-Seq, an RNA-Protein interaction prediction + CISBP, a Catalog of Inferred Sequence Binding Preferences). Presently, we are building a gene regulatory network to machine learn prediction of regulatory and downstream partners of DRG1/2 in *Leishmania.*

As a corollary to our interest in host responses to infections, we have been studying the generation of immunological memory in B-lymphocytes. Interactions between CD40 on B cells and CD40L on T cells are responsible for several aspects of acquired immune responses including generation of memory B cells. We are currently studying the molecular mechanisms underlying the generation of memory B cells, stimulated by CD40 mediated events. In order to gain insights into early events leading to memory B cell formation, we analyzed the genome-wide expression profile of murine naive B cells stimulated in the presence of anti-CD40. We have identified nearly 10000 genes whose expression is altered minimally 1.5-fold at least at one-time point over a three-day time course. The array analysis indicates that changes in expression level of maximum number of these genes occur within 24 hours of anti-CD40 treatment. Another approach to study the events following CD40 ligation, has been to examine the expression of known regulators of naive B cell to Plasma cell transition. The expression profile of these regulatory genes indicates firstly, that CD40 signalling moves B cells to a memory phenotype that is intermediary between the naive and plasma cell stages of the B cell differentiation. Secondly, the major known regulator of plasma cell differentiation, BLIMP1, gets irreversibly down regulated upon anti-CD40 treatment. We also observed that CD40 signalling dominates over LPS signalling, in that even after anti-CD40 is removed from the cells, BLIMP1 continues to be repressed. This observation in combination with our microarray results and our studies on candidate genes encoding chromatin-modifying factors suggests a role for epigenetic events in the maintenance of this memory-like state. Additionally, our data suggests that CD40 signalling mediates BLIMP1 down regulation by non-Pax5/non-Bcl6 dependent mechanisms. Our results also indicate that CD40 signal affects BLIMP1 regulation at a post-transcriptional level.

Currently, we are studying the mechanisms by which CD40 signalling targets BLIMP1 downregulation. We have examined the role of miRNAs in, both the process of Memory generation and the regulation of BLIMP1 transcript. We find that miR26a,23b, 30a, 146, 17 & 150 were down regulated and miR292 & 155 were upregulated respectively in CD40 stimulated B cells. We also find that specific candidate targets, namely, c-MYB, BTLA, PTEN, CCND2 and TRAF6, of our candidate miRNAs also exhibit commensurate changes in steady state level in CD40 stimulated B cells. Using gene set enrichment analysis on our existing Microarray data known/predicted targets of our candidate miRNAs, we observed a significant enrichment of miRNA-targets at 48 hr time point but not at 24 hr post stimulation. Further, gain of function studies in which we ectopically introduced our candidate miRNA into the cells showed a corresponding modulation of target genes, underscoring the functionality of the observed differential regulation of select miRNAs upon CD40 treatment

Additionally, we are extending our previous studies by characterizing the cells post CD40 signalling along various parameters including cell surface markers, isotypes of Ig transcripts and longevity and survival. Cells receiving both stimulants proliferated to a greater degree, underwent more divisions and

survived longer than those receiving only LPS. The co-stimulation also triggered more isotype-switching and expression of memory- (CD80+CD73+) and germinal centre- CD95+PNA+) specific markers, but lower expression of the plasma cell marker, CD138. However, surprisingly, during their extended survival, LPS+αCD40-treated cultures shifted towards being predominantly CD138+, reaching about 75% by 18 days. Furthermore, upon premature termination of signalling to these cells, there was an increase in both, percentage of CD138+ cells as well as median of CD138+ expression, hinting at a transition from plasmablasts to plasma cells. This expression pattern inversely correlated with that of a reported division timer, c-myc, whose repression was not necessarily BLIMP1-driven. These stimulant-deprived cells also displayed a quiescent phenotype and were capable of re-entering the cell cycle when re-stimulated after the hiatus, possibly with CD138– cells dominating the response. Strikingly, the progressive terminal differentiation overlaps with a reduction in the propensity of cells to get re-stimulated, consistent with the "decreasing potential model" proposed for T cells. Our results show that CD40 signalling extends the lifespan of B cells and retards the terminal differentiation triggered by LPS, thereby providing scope for other events like isotype-switching and memory formation. Eventually, this versatility reduces over time or with cessation of signalling. B cells then switch to a default terminal differentiation into plasma cells as they sense the end of their ability to divide further.

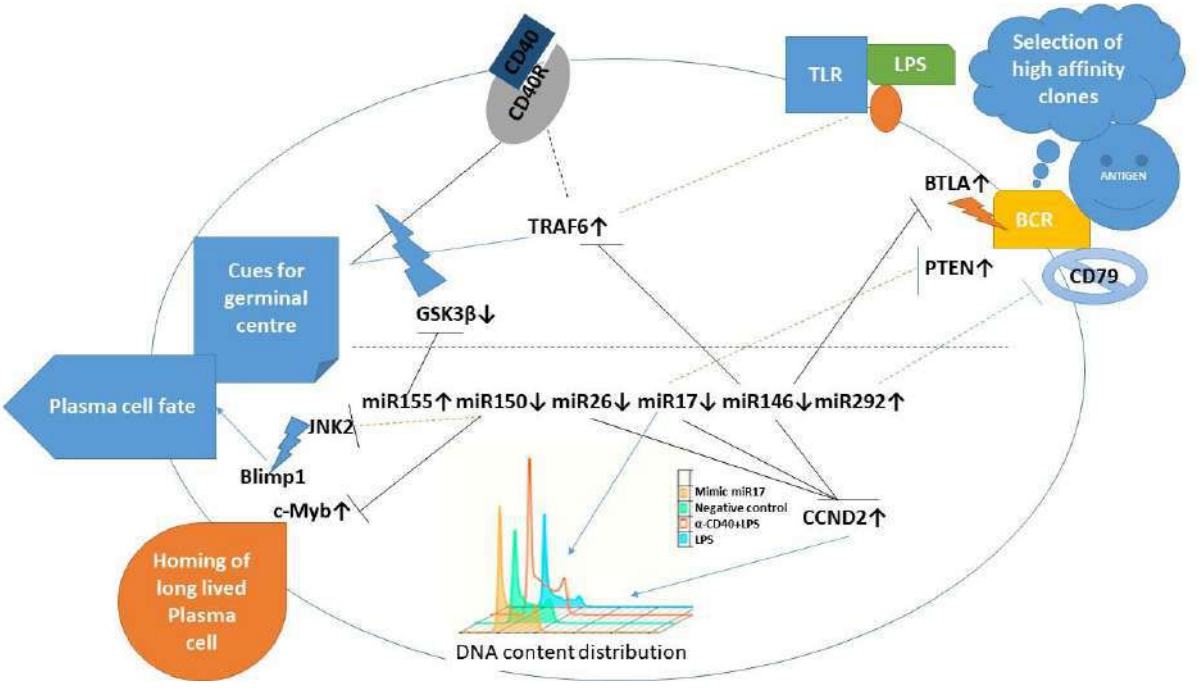


Fig. 1: Coordinated changes in miRNA-mRNA levels induced by CD40 signalling in B lymphocytes during early stages in decision between Plasma vs Memory

KARTHIKEYAN VASUDEVAN

Ecology and Conservation of Endangered Species

From Left to Right (Back row): Anuradha Reddy, Yashvardhan Singh Sengar, Snehalatha Vadigi, Siddharth Bhatia (Middle row) : Krishna Kumanduri, Karthikeyan Vasudevan, Vaishnav, Gayathri Sreedharan, Harika Katakam, Akshay, Ashish Jha (Bottom row) : Afsar Soghra, Ravi Singh, Arjitha, K. Rajyalakshmi, Avni Blotra

RESEARCH INTERESTS:

- Understanding ecology of wildlife diseases and its implications at the community level
- Phylogenetic and population genetic studies on endemic and endangered species
- Bridging gaps and knowledge in snake venomation in humans for better treatment outcomes

"Our research group focuses upon understanding factors that lead to endangerment of some vertebrate species"

Selected recent publications

- Prathap G (2018). New distributional record of the northernmost Myristica swamp from the Western Ghats of Maharashtra. *Current Science* 115: 1434.
- Harikrishnan S, Vasudevan K (2018). Niche dissociated assembly drives insular lizard community organization. *Scientific Reports* 8: 11978.
- Mutnale MC, Anand S, Eluvathingal LM, Roy JK, Reddy GS, Vasudevan K (2018). Enzootic frog pathogen Batrachochytrium dendrobatidis in Asian tropics reveals high ITS haplotype diversity and low prevalence. *Scientific Reports* 8: 10125.

Phylogenetic and population genetic studies

Yellow Throated Bulbul (YTB): This is an endemic and threatened species found in small pockets of suitable habitats in scrub forests across Eastern Ghats, Inland hillocks and Southern Western Ghats. Being endemic to the region with sparse and discontinuous distribution, genetics of YTB populations secure signatures of ancient land form changes and population level differentiation. The populations of YTB across its geographic range were sampled to understand the genetic differences between different populations. The evolution of 24 species of Bulbuls in Indian Subcontinent was also studied. Data shows that YTB has a unique evolutionary history among bulbuls in the Indian peninsula. Population genetic study suggests that the populations are interconnected and it is a panmictic population. Environmental niche model predicts loss of suitable habitat in future (year 2050 and 2070) under various climate change scenarios. With only 10% of predicted habitat falling within the protected area, the threats for the species are growing. It has substantial climatic niche overlap with sympatric species but occupies a different position in morphometric space. A comparative analysis of morphometric and climatic data revealed that sympatric species of bulbuls show fine scale niche partitioning.

Gharial: The National Chambal Sanctuary in India holds the largest, most-stable and most-studied gharial subpopulation in the wild. The Gharial is one among the longest lived reptilian species (up to 100 years) and spends half of its life as a reproductively active animal. Since it has a prolonged pre-reproductive period, there would be no immediate apparent consequences upon the removal of breeding individuals. However the future ramifications would be a dramatic decline in their population size, hence making the study of population genetics of prime importance in Gharials. To study the behaviour aspect of this species, Gharial Ecology Project (GEP) have been catching and tagging the animals. While catching and tagging the animals, GEP team has also collected the scute samples from these animals and these samples were used for genetic studies at LaCONES. We focused on the genetic diversity in Chambal gharial population using microsatellite markers. Gharials are very unique in terms of their communal breeding and parental (especially male guarding) caring behaviours. All these distinctive characteristics raise very interesting questions like, how many adult males sire the hatchlings at one particular nesting site etc. To address this question, we have collected approximately 7000 egg shells from different nesting sites in three consecutive years (2017 and 2018). We are isolating DNA from these egg shells. We have used 100 gharials cutes samples to evaluate the polymorphismexhibited by15 microsatellite markers in the gharial population. After selecting the polymorphic microsatellite markers, these markers will be used on the egg shell DNA with the help of NGS technology. We will reveal factors that determinetheir breeding and parenting behaviours. All the scute samples were used to evaluate the genetic diversity of gharial population in Chambal. We have found 4 monomorphic and 11 polymorphic markers. Average observed and expected heterozygosity was found to be 0.371 and 0.376, this suggests that Chambal population is not suffering from inbreeding. We have also been trying to screen and optimize 24 sets of microsatellite primers given by Dr. Ramesh Aggarwal. We found that 4 sets of primers are working in PCR and giving typical microsatellite peak in capillary electrophoresis. These new markers are being evaluated for their polymorphic status.

Wildlife disease ecology

Tick studies: Ticks are arachnids, typically 3 to 5 mm long and are specialized obligate hematophagous ectoparasites of mammals, birds and reptiles, distributed worldwide.Ticks have four stages to their life cycle – egg, larva, nymph, and adult. Ixodid ticks require three hosts, and their lifecycle takes at least a year to complete.Of 1,415 pathogens known to infect humans, 61% are zoonotic and 70% emerging infectious diseases are of zoonotic origin. Ticks are among the most competent and versatile of vectors, second only to mosquitoes as carriers of human diseases, and are the most important vector of

pathogens affecting cattle worldwide. Global loss due to ticks and tick borne diseases (TBD) were estimated to be between 14 and 19 billion USD annually. Important TBDs affecting humans and livestock in India: Indian tick typhus, Haemorrhagic fever, etc.The diversity-disease relationship explores the impact of loss of biodiversity on disease risk. When an increase in biodiversity leads to decrease in disease risk, it is termed as the 'dilution effect' and when it leads to increase in disease risk, it is termed as the 'amplification effect'. In this study, we use the tick vector and tick borne diseases to explore the relationship between biodiversity and disease risk. We will characterise the microbial diversity present in tick through next generation sequencing. Such analysis is important to understand the nature and dynamics of tick borne diseases.This will help us evaluate "Disease Risk–Diversity Hypothesis". The work will lead to repository of microscopy images along with Taxonomic identification of ticks in wildlife.

Chytrid fungus: Emerging Infectious diseases (EIDs) pose a major threat to both humans and wildlife across the globe. *Batrachochytrium dendrobatidis* (*Bd*) is an emerging aquatic fungal pathogen that seems to be amphibian-specific. *Bd*has distinct stages in its life cycle wherein it has a flagellated zoospore form that initiates the colonization, upon finding a frog skin. Following the establishment on the host, it reproduces asexually in the host epidermis, to release more zoospores, thus repeating the cycle. The thickening of the skin following *Bd* infection thus brings these essential functions like osmoregulation to a standstill resulting in the fatality. There are a few lineages of *Bd* identified from different regions like *Bd*-Cape (South Africa), *Bd*-Brazil (South America), *Bd*-Asia (East Asia), *Bd*-Swiss (North Africa and Europe). *Bd*-GPL is a global panzootic lineage which has been cultured from all areas where mass mortality has occurred. Mass mortality has not been reported in India yet. It has been understood that the haplotypes of this chytrid fungus has insertion-deletion mutations at the Taqman probe binding site of the ITS site of the fungal genome. This is one reason why the specificity of the qPCR assay is poor though it has higher sensitivity than nested-PCR assay which is the current used method in India. A new assay is being developed by referring to the mitochondrial genomes of all the lineages of *Bd*, sequenced so far. The major objectives of this study are: (1) To design a robust and sensitive assay to detect *Bd* using software-ECO-PRIMER.; (2) Quantify infection and recovery rates of *Bd* by swabbing tagged animals.; (3) To identify environmental covariates that influence pathogen dynamics; (4) Place the Indian *Bd* lineage on the global phylogeny of *Bd*. We have used baits of shed snake skin and buccal sheaths of tadpoles as source of chytrid and attempted culturing *Bd.* We have spent 195 days in 2018 and 2019 in the field and have implanted Passive Implant Transponders (PIT) tags in ~900 frogs and isolated DNA from the swabs collected from them.

Fig. 1: Research Implanting Passive Implant Transponders (PIT) tags

Geographical venom variation in distinct populations of vipers

Snakebite claims around 50,000 people in India every year. This may be attributed to many factors like lack of awareness, incompetent antivenom, etc. Intraspecific venom variation has been widely reported is various snake species. This could lead to serious issues with the efficacy of antivenom raised against venom collected from a small region. Similar is the case in India where polyvalent antivenom is made using venom collected by Irula cooperative society based in Chennai and distributed all across the country. *Echiscarinatus* or saw-scaled viper is a

medically important snake in India with no information on venom variation in geographically distinct populations. We performed venomics of *Echiscarinatus* venom (ECV) collected from Goa, Rajasthan and Tamil Nadu. Significant differences were observed in the SDS-PAGE bands indicating variation in venom composition. Functional mapping of toxins revealed differences in symptomatology of snakebites from these regions.

We evaluated Antivenom-venom immune complexes using SEC-HPLC and estimated binding parameters for ECV from different location. We also looked at potency of venom using in-vitro coagulation assay. We estimated clotting times of human citrated plasma using ECV from different locations.

Fig. 2: Researcher extracting venom from saw scale snake in the field of Rajasthan

VEGESNA RADHA

Signaling and Regulation of Cell Fate

From left to right: Ch. Ramulu, Bh. Muralikrishna, V. Radha, Divya Sriram

RESEARCH INTERESTS:

- Regulation of differentiation and cell fate decisions.
- Mechanism of action of cell death regulators

"Focus of work during the year has been on understanding regulation of RapGEF1 expression and localization, and identifying mechanisms involved in RapGEF1mediated cell differentiation. We have also identified the molecular mechanism of cold induced autoinflammation caused by NLRC4 mutant"

Selected recent publications

- Shakyawar DK, Muralikrishna B, Radha V (2018). RapGEF1 dynamically associates with nuclear speckles and regulates mRNA splicing. *Molecular Biology of the Cell* 29: 1111-1124.
- Shakyawar DK, Dayma K, Ramadhas A, Varalakshmi C, Radha V (2017). RapGEF1 shows regulated nucleo-cytoplasmic exchange and represses histone modifications associated with euchromatin. *Molecular Biology of the Cell* 28: 984-995, 2017.
- Raghawan AK, Sripada A, Gopinath G, Pushpanjali P, Kumar Y, Radha V, Swarup G (2017). A disease-associated mutant ofNLRC4 shows enhanced interaction with SUG1 leading to constitutive FADD dependent caspase-8 activation and cell death. *Journal of Biological Chemistry* 292: 1218-1230.
- Begum Z , Varalakshmi Ch, Sriram D, Radha V (2018). Development and characterization of a novel monoclonal antibody that recognizes an epitope in the central protein interaction domain of RapGEF1 (C3G). *Molecular Biology Reports* 45: 1809-1819.

Signaling and regulation of cell fate

Physiological processes such as proliferation, differentiation, cell death and tissue morphogenesis, are dependent on signaling molecules that control gene expression and cytoskeletal dynamics, to decide cell fate. A large number of human disorders are associated with defective signaling and cell fate decisions. Our research focuses on characterizing regulatory molecules of intracellular pathways leading to differentiation and cell death, to understand how these fundamental processes are controlled, and how deregulation results in pathological situations. These studies impact our understanding of normal development and maintenance of adult tissue integrity. Currently, our focus is on the guanine nucleotide exchange factor, RapGEF1, which is ubiquitously expressed and functions in multiple signaling pathways. RapGEF1 regulates activation of small GTPases, Rap, R-Ras and TC10 and is known to have functions dependent on both catalytic and protein interaction domains. RapGEF1 is essential for mammalian embryonic development and many cellular functions in adult tissues. The molecular effectors and function of RapGEF1 in fetal and adult tissues are poorly defined. Studies in our laboratory showed for the first time an involvement of RapGEF1 in signaling to actin reorganization, and its role in differentiation. We have shown that RapGEF1 functions as a negative regulator of β–catenin signaling and may therefore control cell fate. How RapGEF1 regulates differentiation and other cellular functions is not fully understood.

RapGEF1 undergoes nucleo-cytoplasmic exchange, to regulatechromatin modification and mRNA splicing, properties that enable control of gene expression. Nuclear localization of RapGEF1 is regulated by cellular GSK3β activity, and RapGEF1 was identified as a novel interacting partner and regulator of GSK3β activity. During the year, we showed that GSK3β phosphorylates primed as well as unprimed target sites in RapGEF1, and that RapGEF1 regulates GSK3β activity through Akt and PI3K. Molecular modelling and mutational analysis identified a GSK3 β interaction domain in the C-ter of RapGEF1 that overlaps with its NES. Colon cancer cell lines with low expression of RapGEF1, showed reduced Akt activity, and high GSK3β activity. We also showed that RapGEF1 induces myocyte differentiation through its ability to inhibit GSK3β.

Predominant expression of two alternately spliced isoforms of RapGEF1 giving rise to 140 kDa polypeptides are ubiquitously expressed in all mammalian tissues. We identified the expression of a novel, and unique isoform, having additional amino acids in the CNS. This isoform gives rise to a 175kDa polypeptide, seen primarily in adult brain and spinal cord. Examination of the expression of RapGEF1 in different cell types and functional regions of the brain showed predominant cytoplasmic localization of RapGEF1 in neurons, but not in glial cells. Co-localization with DCX and Nestin showed that it is expressed in neural precursor cells. Both the 140, and 175 kDa polypeptides are expressed in embryonic brain, prior to expression of NeuN.

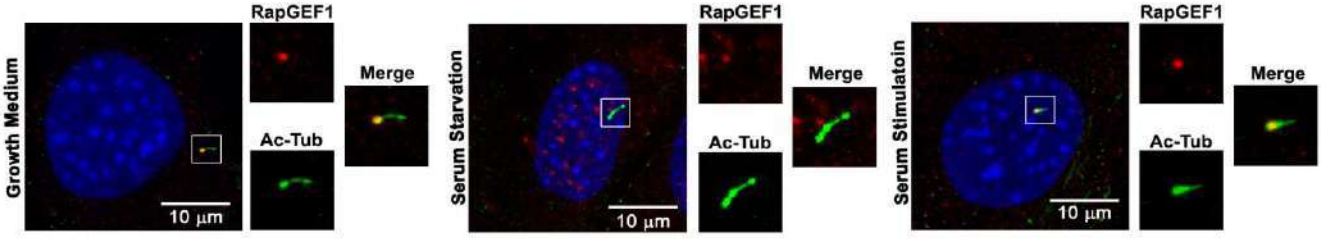
Centrosomes and primary cilia present in all animal cells, enable decisions of cell fate and regulate multiple cellular functions. Key molecular players responsible for centrosome division or formation of primary cilia are largely undefined. RapGEF1 localizes to the mother centriole and functions to regulate centriole division and primary cilia dynamics. Upon cell proliferation arrest by serum starvation, RapGEF1 localization at the centrosome is lost, and moves into the nucleus. Centrosomal localization is regained upon entry into the cell cycle by re-feeding serum (Figure). Cells depleted of RapGEF1 by CRISPR/Cas9 showed supernumerary centrioles and abnormal primary cilia, with an increase in the proportion of ciliated cells, having longer structures. Our results suggested that RapGEF1 functions to regulate centrosome dynamics, and this property may be important for its role as a cell fate determinant during embryonic development. During the year, we examined the consequence of RapGEF1 knockdown in a mouse embryonic stem cell line, TG2A in collaboration with Dr. Chandrasekhar. Stable clones lacking RapGEF1 tended to retain a pluripotent state,

and therefore the role of RapGEF1 in ES cell differentiation is being investigated.

In collaboration with Dr. Ghanshyam Swarup, we have shown that one of the autoinflammatory syndrome-causing mutants of NLRC4, H443P, constitutively activates caspase-1 and that HSC70, and HSP70, two molecular chaperones interact with Ipaf *in vitro* and *in vivo*. During the year we showed that caspase-1 activation by the mutant is moderated by temperature sensitive interaction with HSC70. Exposure to subnormal temperature reduces interaction of H443P with HSC70, causing caspase-1 hyperactivation. This is the first study that provides a molecular mechanism for exacerbation of inflammation induced by cold temperature in individuals carrying the H443P mutation, that causes FCAS.Our attempts to understand how M98K, a mutation in optineurin is defective in signaling, showed that retinal ganglion cells that express this mutant undergo autophagic cell death. Since this mutation is associated with glaucoma, we characterized adult human primary retinal cells grown in culture and show that they express markers of retinal precursor and ganglion cells, and can be cultured for 6 passages. Expression of the glaucoma associated mutants, M98K and E50K, but not wild type, or ALS mutant optineurin triggered cell death in these cells. M98K induced cell death is dependent on activity of TBK, and can be inhibited by Amlexanox. Our study shows that primary cells obtained from adult human retina can be utilized as a model for understanding disease pathogenesis and drug screening/testing.

Highlights and significance

During the year we identified novel properties that explain the essential role of RapGEF1 in regulating cell fate decisions. Significantly, we show that RapGEF1shows dynamic centrosomal localization, and functions to maintain centriole number and cilia length. We demonstrated that in the adult CNS, an alternate longer isoform of RapGEF1 is predominantly expressed, and the form expressed in other tissues is lost during brain development. Our findings explain why loss of RapGEF1 causes early embryonic lethality, and suggest that mutations in RapGEF1 may cause developmental defects called ciliopathies in humans. We have identified heat shock proteins as regulators of inflammasome activity in response to sub-normal temperatures, and elucidated the molecular mechanism of cold induced auto-inflammation caused by NLRC4 mutations. These results enhance our understanding of normal cell functions at a molecular level and how deregulation can cause pathology.



Dynamic movement of RapGEF1 from the centrosome to the nucleus in response to cell cycle arrest: RapGEF1(red) is present at the basal body/centrosome in exponentially growing myocytes (Ac-Tubulin (green) marks the primary cilium), Cell cycle arrest by serum starvation results in RapGEF1 translocating to the nucleus & back to the centrosome/basal body upon re-entry into the cell cycle.

SUNIL KUMAR VERMA

Molecular Biology Applications in Wildlife Conservation and Plant Forensics

From left to right: Akanksha Singh, Vetriselvi PM, Arjita Jaiswal, B Kruthika Prasanna, SP Ratish Prashanth, Chaitanya Vats, Sunil Kumar Verma, Pritam Saha

RESEARCH INTERESTS:

- Signal Transduction in human health and disease conditions
- Molecular Evolution of Genes and Species
- Wildlife Forensics and Plant Forensics

"The focus of my laboratory is to study signal transduction pathways in human health and disease; and applied research in the area of wild flora and wild fauna forensics and conservation."

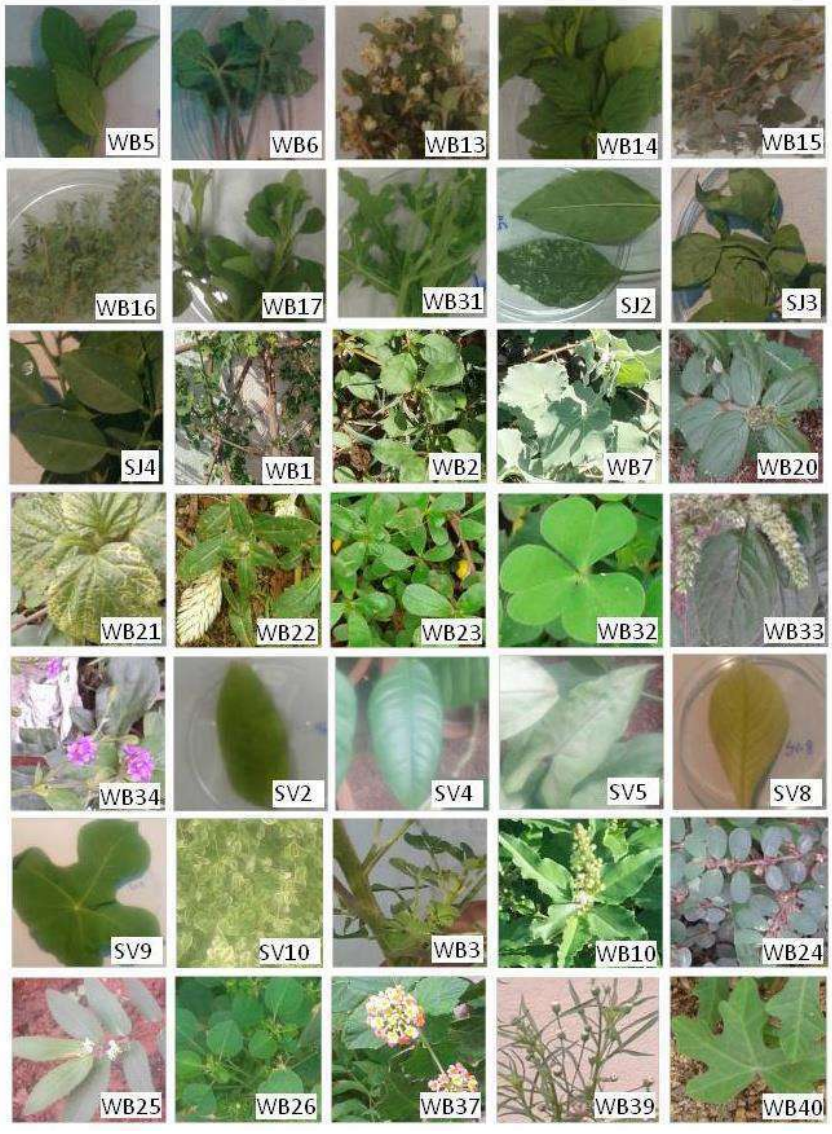
Selected recent publications

- A Revolution in Wildlife Forensics. India Perspectives, 2015, Vol.29, Issue 4, pp 74-75. (A cover "Success Story" by Ministry of External Affairs Govt. of India, published in 16 languages and distributed in 170 countries).
- Verma SK, Sarkar MK (2015). *Decalepis arayalpathra* (J. Joseph & V. Chandras.) Venter, an endemic and endangered ethno medicinal plant from Western Ghats, India. *Natural Product Research* 29: 394-395.
- Verma SK, Goswami GK (2014). DNA evidence: Current perspective and future challenges in India. *Forensic Science International* 241: 183-189.
- Verma SK, Singh L (2003). Novel universal primers establish identity of enormous number of animal species for forensic application. *Molecular Ecology Notes* 3: 28-31.

Medicinal plants based formulations have long been used for the treatment of various human ailments. The unsustainable use of these plants has created a negative pressure on their biodiversity and availability; which also leads to unethical malpractices in modern times by the practitioners and traders of these medicinal plants. It has been presumed that the dramatic increase in the complains pertaining to the toxicity of these plant formulations in present times may also be an indirect effect of these malpractices since what is actually given to the patient may be a spurious product. Therefore, the correct identification and authentication of medicinal plants for their safe use is the need of the hour and needs global attention.

DNA barcoding has emerged as a modern reliable tool for the identification and authentication of individual medicinal and herbal plants at molecular level. This year, the fast track translation (FTT) project aiming to create a reference DNA barcode library of selected Indian flora was continued. Approximately 250 plants were analysed and work was initiated to create their reference database.

In the coming years, this library will be used to establish a DNA based Plant Identification and Forensics Service laboratory in CCMB, which would be a first of its kind of laboratory in India.



Various aromatic herbs analysed in the lab for development of DNA based identification and authentication system.

SREENIVASULU YELAM

Plant Developmental Biology

From left to right: Y. Sreenivasulu, Venkateswara Rao A, Subbaiah, V. Vijaya Bhaskar, G. Bhargavi, P. Malathi

RESEARCH INTERESTS:

- Sporophyte to Gametophyte Transition (SGT) in plants
- Gametogenesis and gamete specification
- Polyembryony in Arabidopsis

"Our group focuses on the signaling pathways that are involved in plant germ cell formation, gametogenesis and gamete specification using Arabidopsis as a model system. The results could serve as possible targets for engineering seeds with new agronomic traits."

Selected recent publications

- Singh SK, Kumar V, Srinivasan R, Ahuja PS, Bhat SR, Sreenivasulu Y (2017). The *TRAF Mediated Gametogenesis Progression (TRAMGaP)* gene is required for Megaspore Mother Cell specification and gametophyte development. *Plant Physiology* 175: 1220-1237.
- Pratibha P, Singh SK, Srinivasan R, Bhat SR, Sreenivasulu Y (2017). Gametophyte development needs mitochondrial Coproporphyrinogen III Oxidase function. *Plant Physiology* 174: 258-275.
- Dogra V, Sharma R, Sreenivasulu Y (2016). Xyloglucan endo-transglycosylase/hydrolase (XET/H) gene is expressed during the seed germination in *Podophyllum hexandrum*: a high altitude Himalayan plant. *Planta* 244: 505-515.
- Dogra V, Bagler G, Sreenivasulu Y (2015). Re- analysis of protein data reveals the germination pathway and up accumulation mechanism of cell wall hydrolases during the radical protrusion step of seed germination in

Podophyllum hexandrum – a high altitude plant. *Frontiers in Plant Science* 6: 874.

- Kaur D, Dogra V, Thapa P, Bhattacharya A, Sood A, Sreenivasulu Y (2015). *In vitro* flowering associated protein changes in *Dendrocalamu shamiltonii. Proteomics* 15: 1291–1306.

Over the past decade, plant sexual germ cell formation has attracted the attention of many scientists, because it includes the transition of a somatic cell to reproductive cell by reprogramming. This transition process is ideally suited to address fundamental questions in plant reproductive developmental biology because:

- Mitotically divided adult somatic cell transforms in to a germ cell which undergoes meiosis to result in a functional meiocyte/meiospore for further gametogenesis.
- During gametogenesis, a few rounds of mitosis and cellularization lead to the specification of four functionally distinct cell types (two synergids, one egg cell, a central cell and three antipodals) all derived from a single spore,
- It helps us to understand the molecular mechanisms that determine sexual fate decisions which is a pre-condition for the targeted manipulation of plant reproduction for agricultural use and crop improvement.

Though literature is available on a number of mutations affecting various stages of reproductive development, the molecular mechanisms that coordinate the germline specification, gametogenesis and seed development mechanism in plants are as yet only poorly understood. In my laboratory, we are committed to identifying the signaling pathways that are involved in germline specification, gametogenesis and gamete specification using Arabidopsis as a model system.

While screening of in-house generated T-DNA promoter trap population of *A. thaliana*, we identified a number of mutants with significant seed sterility. Using the genome-walking approach, the T-DNA insertion sites were identified in these mutants. A BLAST search of the sequence amplified in the genome walk against the *Arabidopsis* genome database showed insertions in tetrapyrrole biosynthesis pathway gene, *TRAF* like gene, nucleotide sugar transporter gene and pectin methylestarase inhibitor (PMEI) gene. These genes are specifically expressed during different stages of female gametophyte development beginning with initiation of germ cell, gametogenesis and gemete specification. Hence, our lab is working to unravel various mechanisms of germline specification, gametogenesis and gamete specification in plants.

Our recent studies on the characterization of *AtHEMN*- a *coproporphyrinogen oxidase*mutation in Arabidopsis revealed failure of fusion of polar nuclei in the embryo sac that led to defects in post-fertilization development of the embryo. This is the first report indicating that the tetrapyrrole/heme biosynthesis pathway operates in plant mitochondria (Fig. 1). Its impairment disturbs ROS homeostasis in flower buds and thereby adversely affects male and female gametophyte development in Arabidopsis.

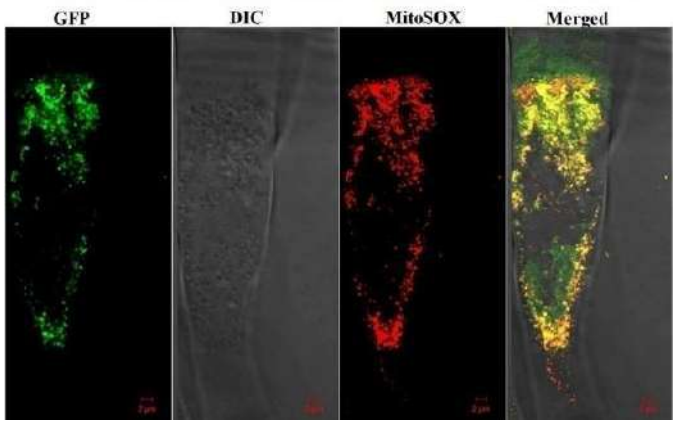


Fig. 1: Cellular localization of AtHEMNprotein in mitochondria. Overexpressing AtHEMNas a function of GFP fused protein in roots of complemented Arabidopsis plants was localized by using confocal microscopy.The same tissue was stained with MitoSOX red for localizing the mitochondria. Co-localization of both GFP and MitoSOX signals in the merged image confirmed AtHEMNexpression in the mitochondria.

We also characterized the role of TRAF like gene, *TRAMGaP* in reproductive development of Arabidopsis. In animals, these proteins regulate diverse cell processes, including immune response, inflammatory response, apoptosis, cancer, embryogenesis, and the survival of the cell itself. In plants, TRAF-like genes have been reported to play a role in pathogenesis (*RTM3*), in abscisic acid-mediated drought stress signaling (*SINA2*), and in insect herbivory. However, no role has been reported so far in reproductive development and this is the first report of the same. The *TRAMGaP* gene is expressed specifically during the development of the male and female sporophytic tissues (Fig. 2). Detailed expression profiling of this gene revealed its role in modulating key genes at various stages of germ cell formation, gametogenesis and during the progression of embryo sac differentiation. Therefore, we have named this as the TRAFMediated Gametogenesis Progression (TRAMGaP) gene. Further analysis of *TRAMGaP*is expected to provide basic information during the transition of sporophyte cell to germ cell.

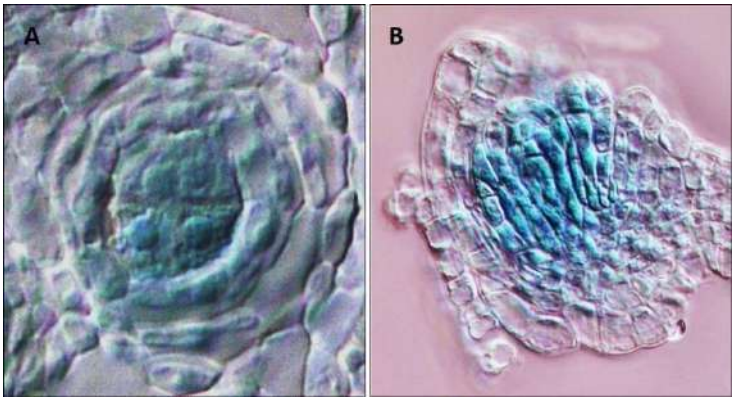


Fig. 2: GUS fused *TRAMGaP* expression in male (A) and female (B) sporophytic tissues of Arabidopsis.

SONAL NAGARKAR JAISWAL

Developmental Biology

From left to right: Sonal N. Jaiswal, Priyanka Pandey, Mayukh B, Aishwarya K, Guru Pratap Singh, Nandan J, Titus Ponrathnam, Shilpa N, Simran G

RESEARCH INTERESTS:

- Regulation of neural stem cell self-renewal, differentiation and quiescence during development
- Neurodevelopmental diseases
- Development of new methods to manipulate fly genes

"We are interested in identifying the mechanisms and the molecular players underlying neural stem cell self-renewal and differentiation. To study these processes, we use *Drosophila* developing brain and human neuronal stem cells as model systems and utilize state of the art genome editing tools and a diverse set of cell and molecular biology approaches."

Selected recent publications

- Ashley J, Sorrentino V, Nagarkar-Jaiswal S, Tan L, Xu S, Xiao Q, Zinn K, Carrillo RA (2019). Transsynaptic interactions between IgSF proteins DIP-α and Dpr10 are required for motor neuron targeting specificity in Drosophila. *Elife* 8: e42690.
- Tan KL, Haelterman NA, Kwartler CS, Regalado ES, Lee PT, Nagarkar-Jaiswal S, Guo DC, Duraine L, Wangler MF; University of Washington Center for Mendelian Genomics, Bamshad MJ, Nickerson DA, Lin G, Milewicz DM, Bellen HJ (2018). Ari-1 regulates myonuclear organization together with Parkin and is associated with aortic aneurysms. *Developmental Cell* 45: 226-244.
- Nagarkar-Jaiswal S, Manivannan SN, Zuo Z, Bellen HJ (2017). Flip-Flop, a facile, cell cycle-independent, conditional gene inactivation strategy differentially tagging wild-type and mutant cells *Elife* 6: e26420.
- Nagarkar-Jaiswal S, Lee PT, Campbell ME, Chen K, Anguiano-Zarate S, Cantu Gutierrez M, Busby T, Lin WW, He Y, Schulze KL, Booth BW, Evans-Holm M, Venken KJ, Levis RW, Spradling AC, Hoskins RA, Bellen HJ (2015).

A library of MiMICs allows tagging of genes and reversible spatial and temporal knockdown of proteins in Drosophila. *Elife* 4: e05338.

Neuronal Stem Cells (NSCs) self-renew to maintain a stem cell pool and give rise to daughter cells that differentiate and generate neurons. NSC self-renewal, quiescence and differentiation are highly regulated processes, any defects in these processes lead to neurodevelopmental disorders such as microcephaly, autism, epilepsy and brain tumor. Therefore, to develop therapeutic approaches to these inherited or acquired disorders, it is important to understand the cellular processes underlying NSC biology. The aim of our lab is to use tractable genetic models to understand the mechanisms underlying NSC self-renewal and differentiation, and apply this information to an evolutionarily conserved system such as human NSCs. To identify novel players involved in NSC maintenance, we utilized *Drosophila* NB, which provide an unparalleled in vivo platform for functional discovery. During my postdoctoral research, I performed an extensive expression-based screen followed by a deGradFP mediated protein knock-down screen on developing brains from fly lines expressing endogenously EGFP-tagged genes. From this screen, I isolated several genes that are enriched in NB, and whose knock-down leads to defects in brain development (Figure 1). These genes are evolutionarily conserved and have been implicated in several neurological diseases such as Down syndrome, Alzheimer's and Parkinson's diseases, but their function in brain development is unclear.

Currently, we are focusing on three genes that were isolated from the screen: (1) *CSN7*, a subunit of COP9 Signalosome, (2) *CG32069*, a homologue of human *Immediate Early Response 3 Interacting Protein 1* (*IER3IP1*) and (3) *CG12050*, a homologue of human *WDR75*. Knockdown of all three leads to a small brain phenotype indicating that these are crucial for brain development. We aim to uncover the roles of these genes in *Drosophila* NB and hNSC maintenance using a series of targeted genetic manipulations.

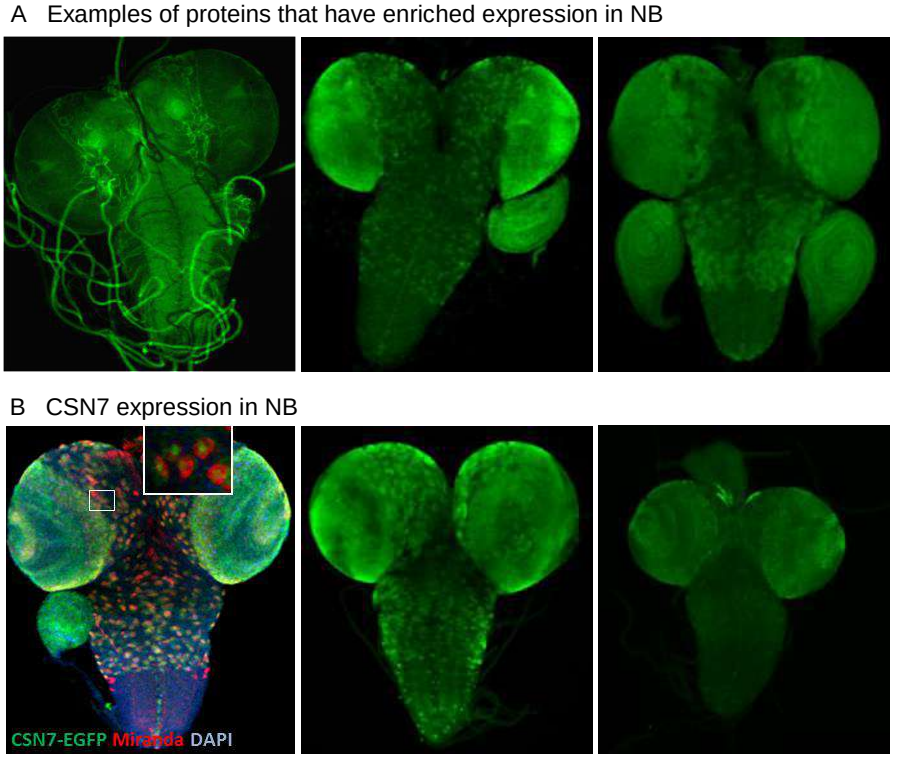


Fig. 1: (A) Mature larval brain from MiMIC lines that show enriched protein expression in NB. Brains are stained with anti-GFP antibody. **(B)** CSN7-EGFP expression in mature larval brain. Inset shows CNS7-EGFP is enriched in neuroblast that are marked with Miranda. **(C)** Age matched brain from control larvae that expresses CSN7-EGFP (left) and brain in which CSN7 is degraded by deGradFP system (right).

PAVITHRA L CHAVALI

Cellular and Developmental Biology

From left to right: Murali Krishna BH, Sagarika Nath, Pavithra L. Chavali, Dhruv Kumar Shakyavar

RESEARCH INTERESTS:

- Mechanisms underlying neurodevelopment and cancers
- Spatio-temporal regulation of neuro developmental proteins
- Role of microcephaly proteins in cancers
- Non canonical roles of centrosomal proteins

"The main focus in our group is to address how molecular mechanisms underlying development are exploited in diseases and infections. We focus on a set of genes whose loss of function lead to babies with small brain phenotype (Microcephaly) and cancers, both of which are phenotypically contrasting pathologies."

Selected recent publications

Chavali PL, Ramachandran R, Chavali S (2019). Functional Categories of RNA regulation in RNA based regulation in Health and Disease, 2019 (Ed: Rajesh Pandey), Elsevier Academic Press.

Research in our group focuses on understanding the holistic role of MCPH proteins in cell cycle during neurodevelopment and cancers. We have now identified that ASPM, the major contributor to global burden of microcephaly and an oncogenic hub in glioblastoma is present in nuclei during interphase. Further this protein is present in nuclear speckles, which are sensitive to Dnase1 digestion, indicating that it could be associated with DNA directly or in a form of a complex. Work is now underway to identify the localisation dynamics of this protein in neural stem cells by endogenously tagging the gene and identifying its interaction partners in different phases of cell cycle (in figure).

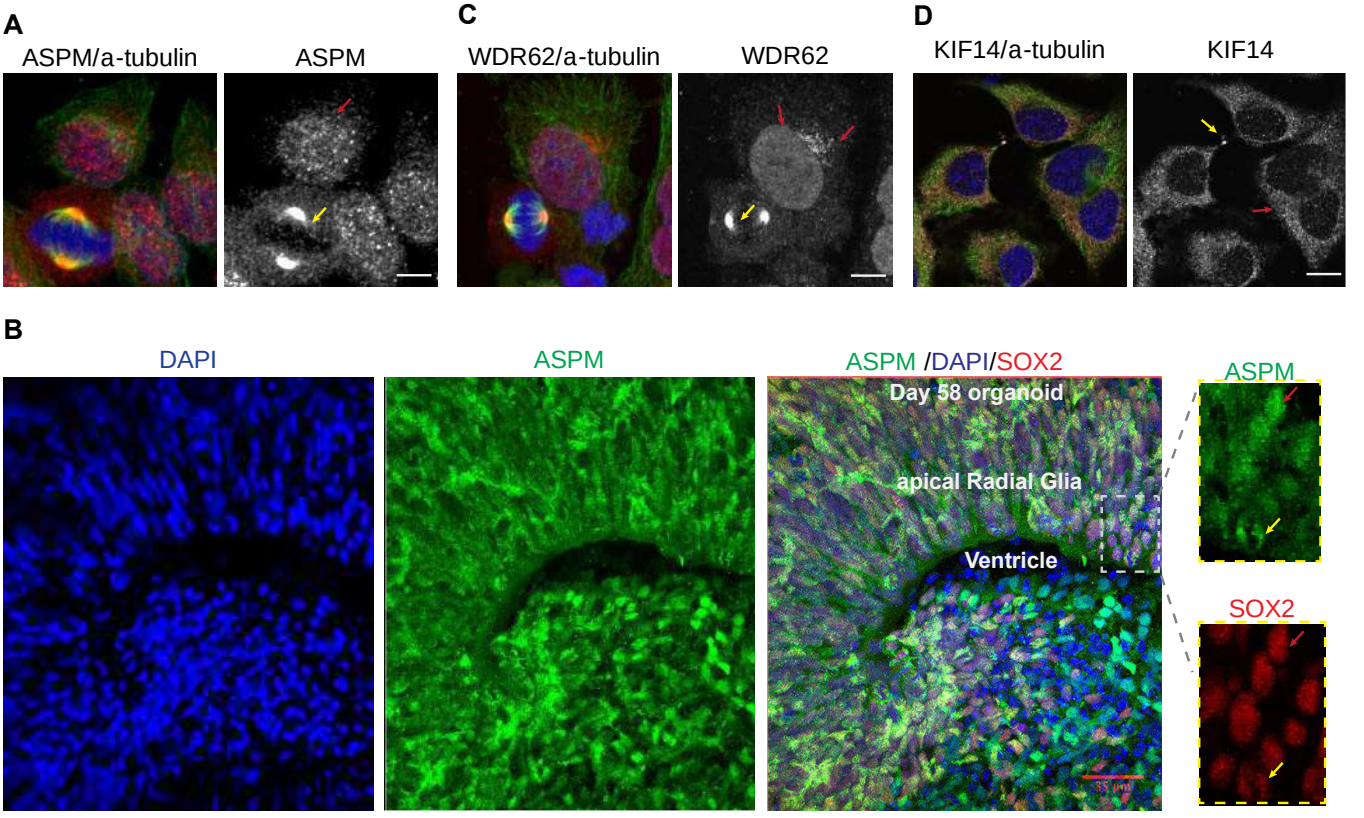


Fig: Sub-cellular localisation of Microcephaly proteins during cell cycle. Immunofluorescence staining of ASPM (antibody courtesy: Dr. J. Bond, University of Leeds, UK) in **(A)** U251MG cells (ASPM: red; α-tubulin: green) and **(B)** section of day 58 cerebralorganoid (ASPM: green; SOX2: red), reveals nucleocytoplasmic staining during interphase and spindle pole localisation during mitosis. **(C)** WDR62 antibody staining in U251 cells reveals a nuclear-golgi staining in interphase and spindle pole staining in mitosis. (WDR62:red; α-tubulin: green). **(D)** KIF14 remains cytoplasmic in interphase and localises to midbodyduring mitosis (ASPM: red; αtubulin: green). Red arrows show cells in interphase and yellow arrows indicates cells in mitosis. Scale bar 8μM **(E)** Normalised protein abundance of three MCPH proteins to actin, after synchronisations of U251MG using double thymidinetreatment.

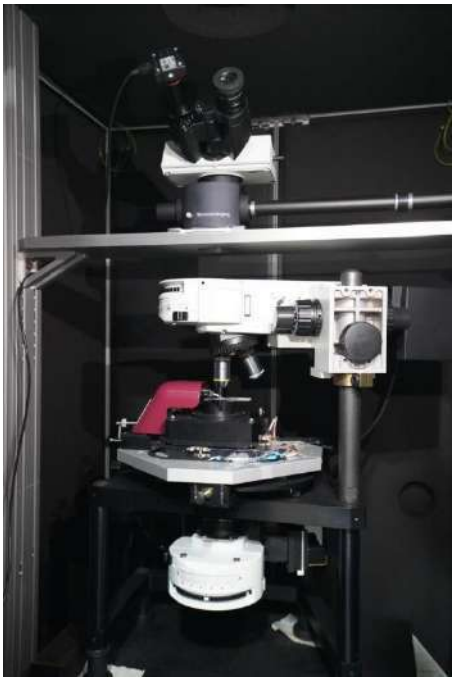
1.1 B
Research Facilities

Advanced Microscopy and Imaging Facility (AMIF)

The AMIF in CCMB is equipped with confocal microscopes, atomic force microscope (AFM), Raman microscope, apotome and regular microscopes.

Olympus FV 3000 Confocal system

This is a new confocal system enabled with 405,445,488,514,561,594,640 nm lasers. Apart from optical sectioning, Fluorescence Recovery After Photobleaching (FRAP), Fluorescence Resonance Energy Transfer (FRET) can be done. Imaging options include tile scans, live cell imaging and long term time lapse imaging.



Zeiss LSM880 with Airy Scan

This is a high resolution confocal system with a 120nm lateral resolution. It is enabled with 405,458,488,514,543,594,633 nm lasers. Apart from optical sectioning, FRAP, FRET, Tile scans, anisotropy, photon counting and live cell imaging can be done.

Leica TCS SP8 system

This is a confocal system enabled with 405,458,488,514,561,594,633 nm lasers. Apart from optical sectioning, FRAP, FRET, Tile scans and live cell imaging can be done.

Atomic Force Microscope

(Nanonics Imaging Ltd Multiview 1000) AFM is a high-resolution scanning probe microscope which measures the forces between the tip of a probe and the sample surface with high sensitivity and gives a topographic image of the sample surface.

Raman Microscope

(RENISHAW InVia Raman Microscope) In Raman microscopy, sample is illuminated with a monochromatic laser beam (532,633and 738 nm) and the resultant inelastic scattered light intensity as a function of frequency shifts is used to construct a Raman spectrum. The chemical composition of a sample can be obtained from the characteristic Raman frequencies.

Zeiss Fluorescence Stereomicroscope with Apotome

This is a high resolution stereo microscope with sectioning along the Z-axis using structured illumination principle. It has zoom ratio of 16. Fluorescence and bright field imaging can be done and it can also be used for 96 well plates. Tile scans can be done and 3D reconstruction is a part of the analysis software.

New Axio Imager Z2 with Apotome

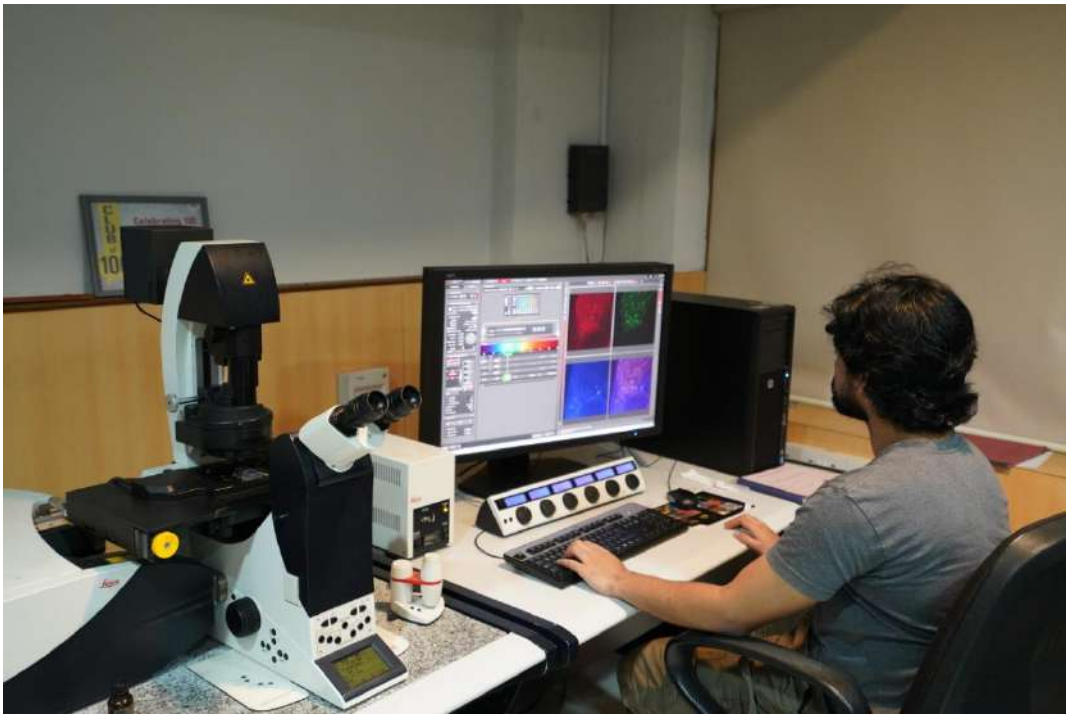
This does sectioning along the Z-axis using optigrid illumination principle and can be used for fixed samples.

Axioimager Z1 microscope

This is a regular fluorescence microscope system fitted with color, black and white camera. It can be used for bright field, differential contrast (DIC), and fluorescence imaging.

Axioplan2 microscope

This is a regular fluorescence microscope. It can be used for bright field, dark field and fluorescence imaging.



Animal House

The CCMB Animal House (AH) was established in 1987 with the main objective of supplying defined strains of mice, rats and rabbits to the scientific community of the centre for research under strict regulation from CPCSEA, Ministry of Environment, Forest & Climate Change, Government of India. All animal house activities are regulated by ONTEXA (Online Indenting System for Experimental Animals) software in which a PI can raise an online animal request as per Institutional Animal Ethical Committee (IAEC) approved project, inventory platform to regulate animal census, mortality, animal production and supply details and monitoring platform to generate the data's of microbial, genetic monitoring along with micro environment parameters of animal rooms. CCMB AH also provides orientation and training to all authorized animal house users to maintain high standards of humane, ethical and responsible use of animals in their research. The AH includes a National Facility for Transgenic and Knockout Animals established in 2002 by a grant from CSIR and DST, and subsequently maintained by core funds. Together, the facility maintains 52 strains of mice including transgenic & knockout mouse models, immuno-compromised (nude) mice, two strains of rats, one strain of hamster and one strain of rabbit. All the mice and rat colonies are housed in individually ventilated caging system (IVCs) where inflowing air is filtered through a hepa filter system imported from Techniplast, Italy. All animal rooms are environmentally controlled and monitored for temperature, humidity and automatically timed 12 hr light dark cycle. The Animal House team comprises of two trained veterinarians and 14 trained staff members who are involved in breeding, management of various lab animals and providing technical support to a variety of ongoing projects. The total number projects approved for animal experimentation under IAECin this year is 127. During this year the below two strains of mice received from Jackson laboratory, USA

Strain Name	Animal Model	Sources
B6.Cg-Tg(SOD1*G93A)1Gur/J mice https://www.jax.org/strain/004435	Model for Amyotrophic Lateral Sclerosis	Jackson Laboratory, USA
B6SJL-Tg(APPSwFlLon,PSEN1*M146L*L286V) 6799Vas/Mmjax mice https://www.jax.org/strain/006554	Model for Alzheimer's Disease	Jackson Laboratory, USA

Number animals supplied during this year are as follows

Mice: 4869 Rats: 129 Rabbits: 24

Cell Culture Facility

The centralized Cell Culture Facility of CCMB caters to the need of all groups in CCMB using cells for their research. The facility maintains a variety of cells for experimental purpose, and provides cell lines, media, serum, plastic-ware and other specific solutions for more than 100 users in CCMB. Experts help in training CCMB staff, students and researchers in cell culture techniques. The facility also serves as a repository for cells, and provides cell lines to various scientific organizations, educational institutions and industries in the country.

The facility is well equipped with laminar flow hoods, CO_2 incubators, inverted microscopes, freezers, cold storage, liquid nitrogen storage facility, FLoid cell imaging system, electroporator & Nucleofector, automated cell counters, photo-dynamic therapy instrument, hypoxia chamber among others. A dedicated BSL2 facility is available that permits use of reagents/viruses/human primary cells requiring biosafety measures. The staff is well trained in maintenance of cell lines, stem cells, organ explant and primary cultures, cell fusion to produce monoclonal antibodies, DNA transfection to establish stable clones and cryopreservation of cells. More recently, platforms for generation of brain organoids have been established. Staff also provides technical help to facility users from various groups in CCMB, as and when required. Around 150 different cell lines are at present being maintained in the facility and are validated to be free of contamination. A short term training course on Animal Cell Culture for students/faculty members/researchers from universities/institutes/industry interested in learning cell culture techniques has been introduced this year on.

DNA Microarray Facility

Microarray is a high-throughput technique for analyzing expression levels of thousands of genes or genotyping large numbers of SNPs in a single experiment.

The microarray facility is equipped to do genome wide analysis with applications in basic research as well as in biomedicine and agro biotechnology. Microarrays (also known as DNA/gene chips) are generated by a technology that integrates molecular biology and information technology.

The facility has Affymetrix GeneChip System for analyzing Affymetrix Chips and the Illumina HiScan System for sensitive and accurate imaging of Illumina Bead Arrays for gene expression, genotyping, and DNA methylation. The entire microarray facility is housed in a dust free room at CCMB main building. The applications that have been used are largely in the areas of gene expression analysis, micro RNA profiling, and genotyping. Gene expression studies have been done with mammalian (mouse, rat and human), plant (Rice and Arabidopsis), and insect (Drosophila). Similarly, the genotyping studies have been carried out in the area of human population genetics and disease association studies.

Apart from managing the facility on day to day basis, staff provides assistance and training in wet lab experiments and in *silico* data analysis with respect to differential gene expression on Affymetrix GeneChip Platform for users within CCMB and also to users outside CCMB.

Fly Lab

Drosophila melanogaster, the fruit fly is one of the most studied and highly tractable genetic model organisms due to its short-life cycle, low maintenance costs, conserved biology, and available powerful genetic toolbox. About 60% of the protein coding genes of *Drosophila* is conserved in human and from these genes about 75% are implicated in various diseases. Therefore, fly is effectively being used for studying basic biology as well as understanding molecular mechanisms underlying human diseases.

In CCMB, we have a very well established fly lab. We maintain about 1500 different fly strains. Among these, we have strains for ongoing research activities that include studies of body patterning, neural development, behavior, stress, longevity etc. We also have fly stains for *in-vivo* genome editing (CRISPR and MiMIC) and about 100 different tissue specific GAL4 driver lines. In addition, we maintain fly models for various human diseases including cancer, Parkinson's disease, Alzheimer's disease and neurodevelopmental disorders such as microcephaly. These strains can very well be used for drug screening. The main fly lab is equipped with several stereomicroscopes for fly pushing, a fluorescent stereo microscope for transgenic larva/fly sorting, and an Axioplan.

In addition, we have a well-established injection facility, which is being used extensively by the research groups from CCMB and CDFD. We also have a fully equipped behavior room with Drosophila Activity Monitoring (DAM) system, T-maze, an equipment used to study learning and memory, and set up for tracking larval locomotory behavior.

We have supporting facilities Nectar and embryo collection lab. Nectar supplies fly food in vials, bottles and embryo collection plates. This facility is equipped with an automatic fly food preparation and dispensing machine, hot plate with magnetic stirrers, cold cabinets, hot air oven and RO water system for fly food preparation. This facility also helps in cleaning and sterilizing the bottles & aluminum trays to prevent contamination. Whereas, the embryo collection room is a small nonstop fly reproduction center, which is designed for constant supply of fly embryos. This facility is equipped with large fly cages and collection plates to collect embryos for high throughput experiments.

We also provide service to other universities/ institutes. Students and teaching staff from different national and international universities visit fly lab to get a hands on experience of *Drosophila melanogaster* culture and maintenance. Fly lab also provides flies to different colleges in the city for teaching purpose and various strains to other research institutes in India for research purpose.

Histology Facility

The Histology Facility at CCMB provides the equipment and technical support for producing high quality tissue sections and staining for microscopy. All histological procedures from tissue acquisition, processing, sectioning, and standard histological, and immuno staining is carried out. Our equipment supports both paraffin-embedded and frozen cryo-sectioning. This facility is equipped with the following instruments:

- Cryomicrotome,

- Rotatory Microtome, and

- Wax embedding station

All other small equipments like water bath, centrifuge, and rotatorque are also available in the facility.

The facilities cover the preparation and processing of tissues, their cutting/sectioning and staining. The services offered are:

- Tissue processing for paraffin/frozen blocks
- Sectioning of paraffin/frozen blocks
- H and E, Masson trichome, van Gieson, Toluidine blue, oil red and Alcian blue staining of paraffin sections
- Training in general tissue processing and histology study methodologies

This facility supports a wide range of projects of the research groups at CCMB.

NMR Facility

Structural Biology

The 600 MHz narrow bore NMR facility was setup in 2009 to study biomolecular structure and function at the physiological condition in the solution. The facility consists of a 600 MHz narrow bore NMR spectrometer equipped with a cryogenically cooled probe. The enhanced sensitivity of the cryoprobe allows *de novo* 3D structure determination of relatively large proteins (MW > 25 kDa) and nucleic acids as well as their ligand-bound complexes at the physiological condition. During 2018-19, we have upgraded the old AVII console with the latest Avance Neo console. The new console allows us to utilize many state-of-the-art NMR experiments including parallel detection of multiple nuclei and non-uniform sampling. The facility is useful to perform structural studies of dynamic biomolecules that are difficult to crystallize (e.g., multi-domain proteins, majorly disordered proteins). The spectrometer is routinely used to derive biologically relevant conformational flexibility of proteins and nucleic acids *in situ*. Some of the important findings derived from the data generated by the facility are:

1) The solution structure of RDE-4 (*C. elegans)* elucidated structural modifications in both dsRBDs that were responsible for selecting the trigger dsRNA
2) Understanding the RNAi initiation in plants through the solution structure complemented with the structure-based activity assays of DRB4 (*A. thaliana*)
3) The solution structure of Crc (~ 32 kDa and presumably the largest solution structure derived by NMR from India) revealed its non-canonical RNA binding surface responsible for regulating the carbon catabolite repression process
4) Understanding the process of enantioselection to elucidate the mechanism of chiral proofreading during protein translation

Over the years, the 600 MHz NMR has become an integral part of CCMB's research activities and had immensely contributed to numerous projects including studies and design of thermostable lipases, studies on antimicrobial peptides, to study the interaction of intracellular loops of GPCRs with membranes, structure-function relationship of key proteins in *P. falciparum* etc. The data generated by the 600 MHz NMR facility has been used in research articles published from CCMB in several internationally acclaimed scientific journals such as Proc. Natl. Acad. Sci. USA (2010), J. Mol. Biol. (2011), eLife (2013), Biochem. J. (2014), PLoS Biol. (2016), Nucl. Acids Res. (2017).

Micro-imaging and Spectroscopy

The 600 MHz Avance III HD Microimager and Spectrometer is interfaced with a wide bore (89 mm) 14.1 T magnet system. It is equipped with actively shielded micro and mini probes with maximum gradient strength of 150 Gauss/cm, which provides *in vivo* images at micron resolution. The system is equipped with volume coils for *in vivo* imaging and spectroscopic studies with mice and rats. The localized *in vivo* NMR spectroscopy could be carried out from a very small voxel (2X2X2 mm^3) in mice brain. Additionally, the spectrometer is equipped with high resolution triple resonance and broadband probes for detection of X-nuclei (^{13}C, ^{31}P, etc) in solution. The current setup is use to study subtle changes in brain atrophy, and understanding neurometabolites, homeostasis and energetics of excitatory and inhibitory neurotransmitters in different neurological and psychiatric disorders like amyotrophic sclerosis, Alzheimer' disease, Parkinson's disease, depression and addictions. Additionally, the setup is used for the characterization and development of MRI contrast agents.

The microimager/spectrometer caters the requirement of following Groups/users: Dr. Anant B. Patel, Dr. Arvind Kumar, Dr. Jyotsna Dhawan and Dr. A. S. Sreedhar.

Proteomics Facility

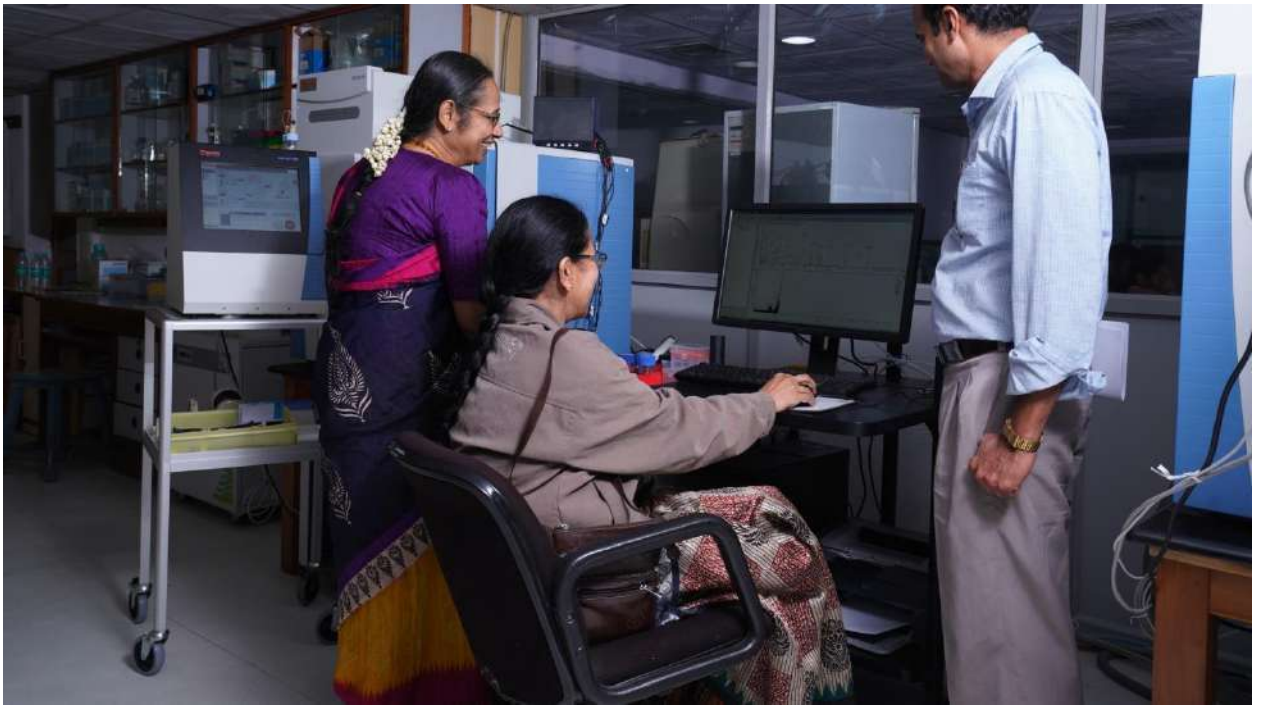
The Proteomics Facility at the CSIR-CCMB provides infrastructure for the identification and characterization of proteins. Mass spectrometry (MS) based proteomics is fast becoming an essential analytical tool for biological scientists. Modern instrumentation and data analysis software can identify and quantify hundreds or thousands of proteins from complex biological mixtures such as cell lysates or body fluids. At CSIR-CCMB, we are equipped with state-of-the-art chromatography systems and mass spectrometers for LC-MS and LC-MS/MS, with a wide range of bioinformatic tools for data interpretation and evaluation. The facility provides a range of services, including:

- Intact molecular weight measurement of proteins
- Protein identification from gel bands
- Protein identification from complex mixtures
- Identification of post-translational modifications
- SILAC, iTRAQ, and label-free quantification of peptides and proteins

Our instrument platforms include cutting-edge Q-Exactive-HF, Q-Exactive, Orbitrap Velos, and MALDI TOF/TOF mass spectrometers, coupled to ultra-high performance EASY-nLC 1200 Systems.

We also have multiple High Performance Liquid Chromatography (HPLC) instruments. These analytical instruments are routinely used for separation and quantification of mixture of proteins/chemical compounds derived either from natural products or synthetic processes. HPLC-facility offers viable solutions due to vast choice of stationary phases and mobile phase options. The different modes and choice of detectors allows analysis of wide range of samples.

In addition to catering internal users in CSIR-CCMB, We provide mass spectrometry-based proteomics services to external users including many Government-funded or Private research labs as well as to Biotechnology industry.



Radio Isotope Facility (CRIF)

Radio isotope is one of the imperative tools in biological research. The radio isotopes are used as a tracer in the biological reaction. Researchers can label and trace the bio molecules by using radio labeled precursor molecules of their interested reaction. CCMB is one of the major users of 32P labeled nucleotides to label DNA and RNA.CCMB also uses other radio isotopes of Hydrogen (3H), Carbon (14C), Iodine (125I), Calcium (45Ca), Chromium (51Cr), Sulphur (35S), and Zink (65Zn) in the form of labeled molecules and bio-molecules. The radio isotope facility works under the guidelines of Atomic Energy regulatory Board (AERB).The facility ensure and monitor the safety of the environment, user and the general public. The radio isotope users are quarterly monitored by personal monitoring system (PMS) to ensure that the users are in the safe exposure level. The facility also manages the radioactive waste management. The radioactive wastes are collected periodically and disposed as per the guidelines of the AERB.

X-RAY Crystallography

Structural Biology X-ray facility provides state-of-the-art resources to elucidate three dimensional structures of macromolecules and their complexes at atomic level. It is equipped with powerful microfocus rotating anode generators: 1) MicroMax™ 007 HF (Rigaku) Cu anode generator with Mar345-dtb image plate detector and Oxford cryosystem 2) FR-E+ SuperBright (Rigaku) dual wavelength Cu/Cr anode generators with R-axis IV++ image plate detector and X-stream cryosystem. FR-E+ system is the most intense home lab source available today for macromolecular crystallography, with focusing optics that can deliver a flux comparable to second generation synchrotron beamlines. Data collected from single crystal diffraction is processed using crystallographic computational software. Molecular-modeling studies are performed using Intel Quad-Core windows and Linux-based workstations, Silicon Graphics (SGI-Fuel) workstations and software that are installed on CCMB server.

High Throughput (HT) Crystallization

A state-of-the-art HT-Crystallization facility provides automation of the complete crystallization set-up. Three major components operational are: (i) Alchemist for liquid handling, (ii) Crystallization robotic systems: Mosquito, Oryx 4 and Hydra II-eDrop for crystallization drop setting and (iii) Minstrel III along with two incubators (4ºC and 20ºC) automated for incubation, storage and inspection of plates for crystal growth.It is supported by dynamic light scattering (DLS), which is a useful tool to diagnose size distribution, stability, and aggregation state of macromolecules in solution prior to crystallization.

For details:
http://www.ccmb.res.in/index.php?view=crystallography&mid=154&id=41

Small Angle X-ray Scattering SAXS

X-ray facility is also equipped with in-house Small Angle X-ray Scattering (SAXS) System for deciphering physical and structural features of macromolecules in solution. SAXS allows to probe size, shape, quaternary structure and complex formation of molecules without crystallization. It helps in understanding (i) structural parameters [radius of gyration (R_g), maximum Dimension (D_{max}), partial-specific volume (V_p) etc], (ii) dynamics of molecules, and (iii) generation of low-resolution shapes of macromolecules.

SAXS facility houses two systems: 1) S3-MICRO Point-Focus system (Hecus X-ray systems, GmbH) with a 50W X-ray source and a Pilatus-100K detector covering a SAXS range between 2000Å and 10Å. 2) BioSAXS-2000 (Rigaku) with 2-D Kratky collimation, mounted on the existing left port of MicroMax™ 007 HF (Rigaku) Cu anode X-ray generator. It is equipped with OptiSAXS Confocal Max-Flux (CMF) for higher brilliance at the sample position and data collection times in the range of minutes. The configuration incorporates an Automatic Sample Changer for unattended overnight operation and an Automatic Analysis Pipeline based on ATSAS package from EMBL Hamburg.

For details: http://www.ccmb.res.in/index.php?view=x-rayfacility&mid=154&id=43

Several structural biology projects that are carried out at CCMB and other research institutes / universities outside CCMB are handled atthese facilities.

Zebrafish Facility

State of art Zebrafish facility is equipped with large scale breeding & embryo collection capacities, live feed (artemia) hatching facility and centralized air facility. Advanced automated stand alone systems maintain lines for developmental biology, cell biology and behavioural biological studies. The facility also houses high end microscopy and imaging system that has motorized advanced stereo fluorescence for multichannel fluorescence and bright-field imaging of zebrafish. The facility is equipped with micromanipulation systems and trained staff to help researchers generate transgenic fishes. A computer aided tracking system (Danio vision with Ethovision software) is available to conduct research on behavioural aspects of the vertebrate model.

1.1 C
Research Resources

Instrumentation

CCMB has a strong and highly supportive Instrumentation Group which takes care of the installation, maintenance and repairs of Instruments. All maintenance and repairs are carried out in-house and no maintenance contracts are given. Maintenance of UPS and audio-video projection systems is also taken care by the group. The group conducts training programs, on the usage of instruments with safety instructions, for the new research students during August every year, for the summer students in May and for the other research staff throughout the year. The state-of-the-art facilities are managed, maintained and run without much down-time due to the support and services provided by the group. Further, the group carries out in-house design, development, modification and fabrication of instruments as and when needed and also provides technical advice to other institutes in the procurement and usage of scientific instruments. The group is also involved in the Young Innovators Program where young school children are taught designing small experiments in electronics and physics. The group's contribution to symposia, seminars, workshops and other events are multifarious, particularly for audio-video and exhibition arrangements.

New equipment installed during the year

- Novaseq 6000 Sequencing system
- High performance 600 MHz NMR spectrometer model Avance NEO
- High sensitive spectral confocal microscope
- High speed flow cytometry, Model - FACS Aria Fusion
- Phosphor Imager ModelAmersham TYPHOON IP
- Infrared Western Blot Imaging Scanner model SAPPHIRE NIR
- NGC Quest 10 Chromatography system
- Plant growth chamber model AR100L3
- Olympus research grade fully motorized inverted microscope.
- High end fully motorized binocular microscope.
- MSM 400 Yeast tetrad dissection microscope.
- Gentlemacs Octo Dissociator with heaters
- Percellys Evolution super homogenizer
- Nanophotometer
- Fragment Analyser TM Automated CE system
- Vibrating blade microtome Leica VT12005
- Mouse myocyte isolation apparatus
- Upgradation of existing Carl Zeiss Metafer system.
- Motorized micromanipulator system
- Raster plotter FPM 25000

Fine Biochemical

CCMB finebiochemicals facility maintains and stocks large number of biochemicals for the ongoing research activities of the laboratory. The facility has a walk-in freezer (-18^{0}C to -20^{0}C) and a cold room. In addition, it also has a freezer (-20^{0}C) for storage of chemicals as per the recommended storage conditions. However, the chemicals stable at room temperature are kept in a room (72 sq. mtrs plinth area) where temperature is maintained at 26--28^{0}C. The stocks of fine biochemicals include amino acids, proteins, enzymes, purification kits and buffer reagents. In addition, it houses stocks of restriction enzymes, antibodies, reagents necessary for purification and detection of recombinant proteins, reagents for DNA/protein gel electrophoresis, PCR, RT-PCR. The requirement for these chemicals is monitored such that procurement is carried out on a regular basis, so as to maintain a constant level of supply. Requirement for these chemicals/enzymes is monitored with a help of software developed by CSIR-CCMB's IT Group. Availability of various chemicals can be seen on CSIR-CCMB's intranet.

Recently, a software tool was developed by CSIR-CCMB's IT department which automatically sends emails to the scientist as soon as the availability of chemicals is updated in the system. It also helps in timely procurement of chemicals/enzymes by giving alert as soon as their availability falls below a threshold limit. It can be accessed on CSIR-CCMB's intranet by all the researchers.

Fine biochemicals indented by all scientists are (652 Purchase orders, with 1293 items) received by fine biochemicals facility, and issued to the corresponding groups in addition to the general chemicals maintained by this facility. 720 consignments were received and 888 invoices were received, verified and forwarded to stores for further action. 4272 items were issued to researchers during 2017-2018 which include fine biochemicals maintained and individual orders.

Information Technology Group

The Information Technology group plays a major role in administering IT infrastructure & services, facilitating scientific collaboration, assisting scientists in creation of computing facility required for R&D projects, and protect network and research data against cyber attacks. The team also develops online applications and tools to help organisation automate and manage the activities of R&D Facilities, Administration, Accounts, Stores & Purchase, Dispensary, etc.

Internet access is through 1Gigabit leased line connection from National Knowledge Network and 10 Mbps leased line connection is also available as redundant. CCMB has a high speed network backbone of 10 Gbps with switched 1 Gbps connection and campus wide wireless connectivity.

IT group also manages High Performance Computing cluster with peak performance 5.525 TF, used for Next-generation sequencing (NGS) research activities and Centralized Network Storage that is mounted to instruments, servers and desktop. Implementation and maintenance of other facilities like surveillance camera, fire alarm system, and telephones are also taken care.

Complete IT infrastructure and services are managed by IT team without AMC support.

For more details: http://www.ccmb.res.in/itgroup/

Laboratory Technical Services

The Lab Technical Services (LTS) in CCMB, CRF (CCMB annexe 2), Uppal, and LaCONES, Attapur, acts as a bridge between the scientific staff and the engineering services. Thus it is the single contact point for scientific staff for all their needs that require involvement of engineers.

This section is headed by an engineer, and some of the major services for which LTS is responsible are : (i) Housekeeping, (ii) Manpower supply, (iii) general maintenance like civil, electrical etc., of laboratory buildings (iv) maintenance of lifts, (v) Pest control services (vi) Horticulture, (vii) maintenance of fire extinguishers (vii) arrangements for scientific and other conventions.

Rajbhasha Unit

This unit helps the institute mainly in complying with various provisions of Official Language envisage by the GoI. It provides training to the officials in Hindi, Hindi typing & stenography and also conducts Hindi workshops to its employees at regular intervals. This unit helps scientists in bringing out the papers, articles, reports in Hindi. This unit also ensures issue of official documents in Hindi as per the OL Act Provisions. This unit also facilitates issuing of press releases in Hindi.

For the past 20 years Rajbhasha unit is bringing out a popular science magazine in Hindi viz., Jigyasa dedicating every issue to a special topic of life sciences. English is being used internationally, for the spread of science extensively for a long time. But in Indian circumstances, use of regional language is must to reach out to the common public to make them aware of scientific developments taking place around them. The main aim of publishing Jigyasa is to popularize and disseminate science among the common public and students in their own language. The upcoming issue of Jigyasa will comprise of latest articles in the field of OMICS. We have a reason to be proud that the articles published in these issues are mostly written in Hindi by our own scientists of CCMB. This act of contributing articles to Jigyasa helped in inculcating a habit of writing regularly in Hindi among our scientists, thus enabling them to fulfill their responsibility towards the society.

This unit of Rajbhasha conducts every year "HINDI DAY" on 14th September and various Hindi competitions and programmes are organized on the occasion. This year Hindi Fortnight has been conducted from 03 Sep 2019 concluding the valedictory function on 14th September, 2019. The winners of the competitions were given away prizes, and cash awards were given to the officials who are doing their official work in Hindi and contributing their part for the implementation of Hindi in the organisation. Former IFS Shri P.K. Sharma, presently working as an advisor and Course Director in Marrichanna Reddy Institute, Hyderabad was invited as Chief Guest of the Function. Every year, we invite some eminent writer, poet or expert of a subject of general interest to deliver a popular lecture in Hindi. This helps our staff and students to interact with such personalities and get benefited by listening to their valuable views.

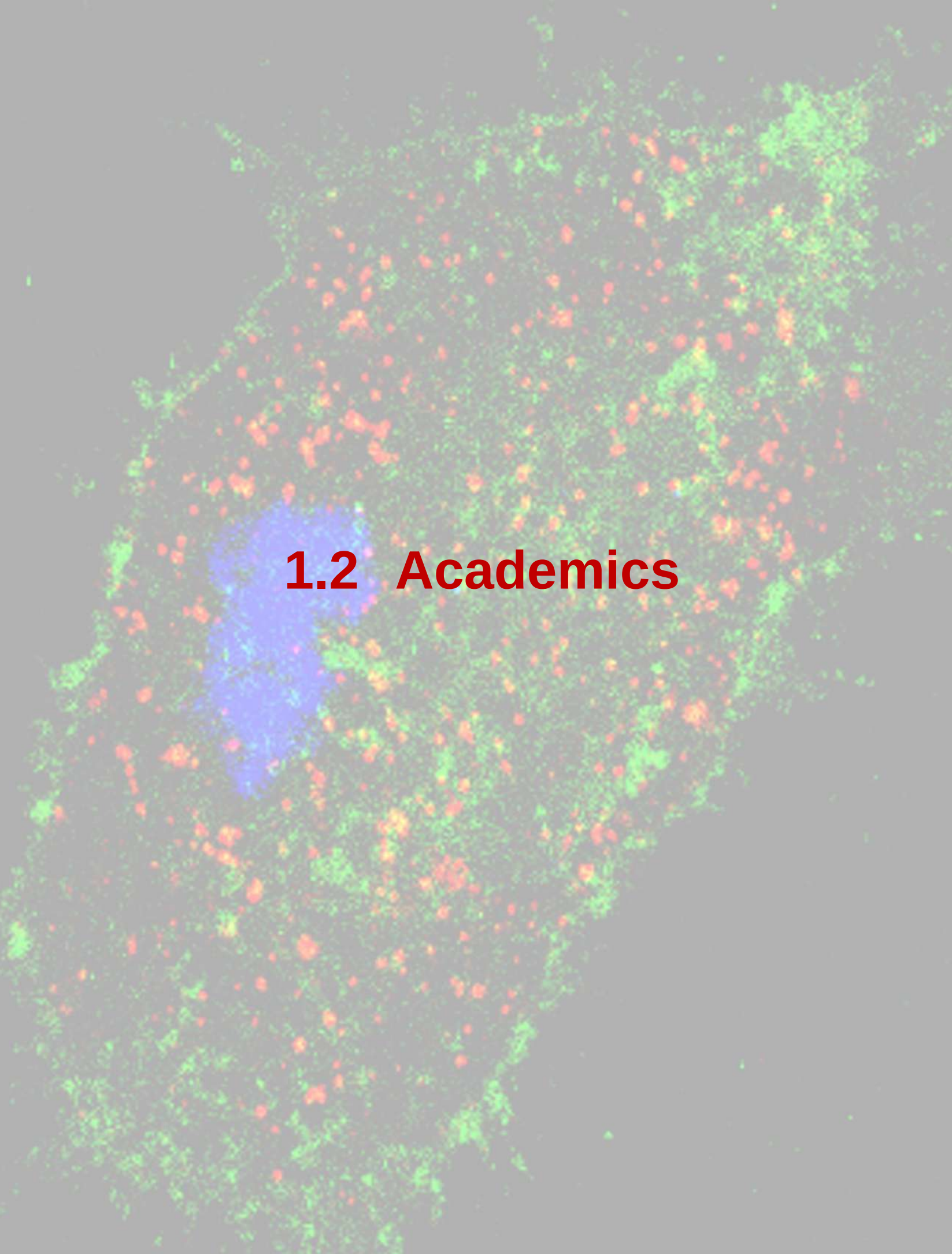
The unit provides opportunity to students and staff to showcase their cultural and literary talents by organising a programme named 'Pratibha'. Its main aim is to provide a platform to the inherent talents of research students and staff at CCMB. The programme is held annually, usally in the month of June. The programme mainly includes literary and cultural activities.

The unit also conducts other activities, viz., inviting eminent speakers of various fields to deliver popular talks in Hindi for the benefit of staff and research students. The spectrum of topic includes space technology, geology, management skills, classical music etc., and they proved very useful for the staff to gain some basic knowledge in these areas.

The Rajbhasha Unit has a very good library consisting 2775 Hindi Books on various subjects viz., classic works of Hindi literature, science, translations and books of general interest. This year 81 books have been added to this collection. The Rajbhasha unit, thus, takes care of CCMB in respect of implementation of Official Language as prescribed by GoI from to time.

Engineering Services

CSIR-CCMB has a number of modern laboratories including the Annexe-1, LaCONES at Attapur and the Annexe-2, Medical Biotechnology Complex at Uppal. Facilities such as workshop, centralized air conditioning, class 10K clean rooms, bio-safety level III labs, ultra low freezers, walk-in cold rooms, walk-in freezer rooms, piped gas supply, pre-treated water supply, liquid nitrogen, 3000 kVA back-up generator sets, 33 kV and 11 kV HT sub stations, distribution system, 30 kW solar power unit, etc. are operated and maintained by the engineering staff.



1.2 Academics

1.2. A Academic Cell & PhD Program

CSIR-CCMB imparts training to doctoral students in an academic program linked either to Jawaharlal Nehru University (JNU), New Delhi or Academy of Scientific and Innovative Research (AcSIR). The PhD program is run by an Academic Cell, which consists of two Academic Coordinators and an assistant. This cell handles almost all the academic activities related to PhD students, including selection and recruitment of students, course work, lab allotment, Doctoral Advisory Committee (DAC) meetings, Comprehensive Exam, and PhD thesis submission. The Academic Cell keeps records of the performance in course work, progress reports of the PhD work, and all AcSIRrelated documents. All administrative matters of the JNU-CCMB PhD program are dealt by a separate JNU-CCMB committee.

CCMB-PhD program targets students who intend to pursue research oriented careers in interdisciplinary areas within or outside academia. Our main goal is to provide students a strong technical background, enhance their capacity for analytical thinking, and address new kinds of problems for the advancement of science and society.

CSIR-CCMB selects candidates for the PhD program in August and January. Eligible candidates are invited to apply and selected based on performance in a written test, followed by two rounds of interviews at CSIR-CCMB. The students can apply through CCMB-JNU, CCMB-AcSIR and CCMB-JGEEBILS streams. 27 students joined for August 2018 and 13 students joined for January 2019 PhD programs. 8 students gave their PhD colloquia and 18 students submitted PhD thesis during April 2018 to March 2019. 18 students have been awarded PhD degree from JNU/AcSIR during this academic year.

1.2. B PhDs Awarded

1. Pravin Kumar Mishra
   Exploring Cerebral Metabolism in Depression by using 13C Nuclear Magnetic Resonance Spectroscopy
   Guide: ***Dr. Anant B. Patel***

2. Meraj Ahmad
   Identification of Novel Genes Associated with Type 2 Diabetes and Related Traits and Understanding their Functional Relationship
   Guide: ***Dr. G.R. Chandak***

3. D V S Sudhakar
   Male infertility: clinical, genetic and functional approaches
   Guide: ***Dr. K. Thangaraj***

4. Komal Ishwar Pawar
   Elucidating proofreading mechanisms involved in correction of mischarging caused by Alanyl-tRNA synthetase
   Guide: ***Dr. R. Sankaranarayanan***

5. Tushar Ranjan Moharana
   Protein engineering of lipase for substrate specificity and stability
   Guide: ***Dr. N. Madhusudhan Rao***

6. Titus Surendra Ponrathna
   Non-homeotic Functions of Hox Genes: Insights from Autophagy and Leukemia
   Guide: ***Dr. Rakesh K. Mishra***

7. M S Ram
   Studies on reproductive and genetic consequences of habitat fragmentation on endangered lion-tailed macaque populations in the fragmented rainforests of Western Ghats
   Guide: ***Dr. G. Umapathy***

8. K. Bipin Kumar
   Transcription factors in innate immune responses of rice
   Guide: ***Dr. Ramesh V. Sonti***

9. N. Suraj Singh
   Elucidating the Genetic Mechanism of Fuel and Nutrient Mediated Fetal Programming of Intermediate Traits related to Metabolic Diseases
   Guide: ***Dr. G.R. Chandak***

10. Richa Khanna
    Characterization of ubiquitin proteasome pathways upregulated by laminopathic mutations
    Guide: ***Dr. Venna K. Parnaik***

11. Dhruv Kumar
    Studies on regulation and nuclear functions of C3G
    Guide: ***Dr. V. Radha***

12. T Apuratha pandiyan
    Genomic Approaches to Cold Adaptation: Importance of DNA repair pathways and RNA metabolism in the Antarctic Pseudomonas sp Lz4W
    Guide: ***Dr. N. Madhusudhan Rao, Dr. Malay K. Ray***

13. Vidhya Krishnamoorthy
    Role of HECW2 E3 ubiquitin ligase in ubiquitination pathways induced by laminopathic mutations
    Guide: ***Dr. Veena K. Parnaik***

14. Akhouri kishore Raghawan
    Mechanisms of signal transduction by NLRC4, a mediator of innate immune response and cell death
    Guide: ***Dr. Ghanshyam Swarup, Dr. V. Radha***

15. T Nandita
    Role of Ubiquitin Proteasome system in Plasmodium biology and pathogenesis
    Guide: ***Dr. Puran Singh Sijwali***

16. Anand Kumar Sharma

Role of Secretagogin, a Calcium Sensor, in Insulin Signaling: Interaction, Regulation and Implications

Guide: ***Dr. Yogendra Sharma***

17. Parna Saha

Genome organization and chromatin landscape in regulating gene expression

Guide: *Dr. Rakesh Mishra*

18. Manish Johri

Role of mechanistic target of rapamycin (mTOR) pathway in Hepatitis C virus (HCV) infection

Guide: ***Dr. H H Krishnan***

1.2. C Training Programs

Dissertation Research Training Program

The Dissertation Research Training Program (DRTP) is an interdisciplinary training program for graduate students from any field of life sciences to do six months to one year research project at CSIR-CCMB under the supervision of a scientist towards their partial fulfillment of Bachelor's (B.Tech, B.Pharm, BDS, MBBS) or Master's (M.Sc, M.Tech, M.Pharm, MD) degree. In this program, in addition to routine laboratory training, candidatesare exposed to recent research developments, scientific ethics, good laboratory practices and career opportunities in life sciences. At the end of the training, candidates present their work in the form of posters to the scientific community at CSIR-CCMB. The program is formalized under skill initiative in June 2017. From January-December 2018, there have been 115 students who enrolled and carried out project-based trainings for either six months or one year duration.

Summer Training Program

Summer training at the CCMB, one of the best-equipped biomedical research institutions in the country involved in basic and applied molecular biology research, is a sought after academic activity. We received 856 applications and after an extensive and rigorous selection process, 58 students could make it to the programme. In addition, 13 students selected through Indian Academy of Sciences (IAS) programme did summer internship at various CCMB labs. There were 11 other students from IISERs, IITs and few other institutes/ universities that underwent training this period, making a total 82 students this summer at the CCMB.

We make lots of efforts to run this program wherein our main intention is to provide students a real time working opportunity in an active research lab. We also select students from state universities and remote parts of India and give opportunities to these students who never had a research laboratory exposure. To make this programme more vibrant and exciting, talks by CCMB faculties on popular bioscience subjects were organized. The students were given orientation by the instrumentation engineers at the beginning of the programme. In the end, a visit was organized to CCMB Annex-I LaCONES (Laboratory for Conservation of Endangered Species), which is an exciting component of the summer training.

Project-based Research Training

Project-based Training programme, initiated in October 2017 caters to students (rather interested individuals), who wish to carry out research-based training in specific areas, based on research expertise of the various PIs at CCMB. In 2018-19, 30 students enrolled for either a 6-month or 1-year duration. Presently there are 22 project-based trainees associated with various PI labs at CCMB.

Summer Training Program

During the last academic year CSIR-CCMB had initiated its Skilling/Training Programs under the CSIR-Integrated Skill Initiative. This year as well, the Skilling Programs were continued and apart from the courses which have been offered last year such as the Medical Student Research Training Program (MedSRT), Winter research observership programme for the medical students, Wildlife forensics, Cell biology, CRISPR technology, Microbiology, new training programmes related to Recombinant DNA Technology (RECOMB), Proteomics (PROTEO), Stem Cell Biology (STEM), and Bioinformatics (NGS), were introduced with a view to cater to both academia and industry needs.

The training program for medical students- Medical Student Research Training Program (MedSRT) was held in May 2018 and 30 medical students had participated in this. The purpose of MedSRT has been to create an orientation of Medical students (mostly in their 2nd and 3rd years) towards clinical research through lectures and hands-on trainings. The Winter Research Observership programme was also organized for MBBS students, where they get to spend 2-weeks in a PI's lab at CCMB to acquaint

themselves with the research life. Twenty two students from medical colleges across India attended this course this year.

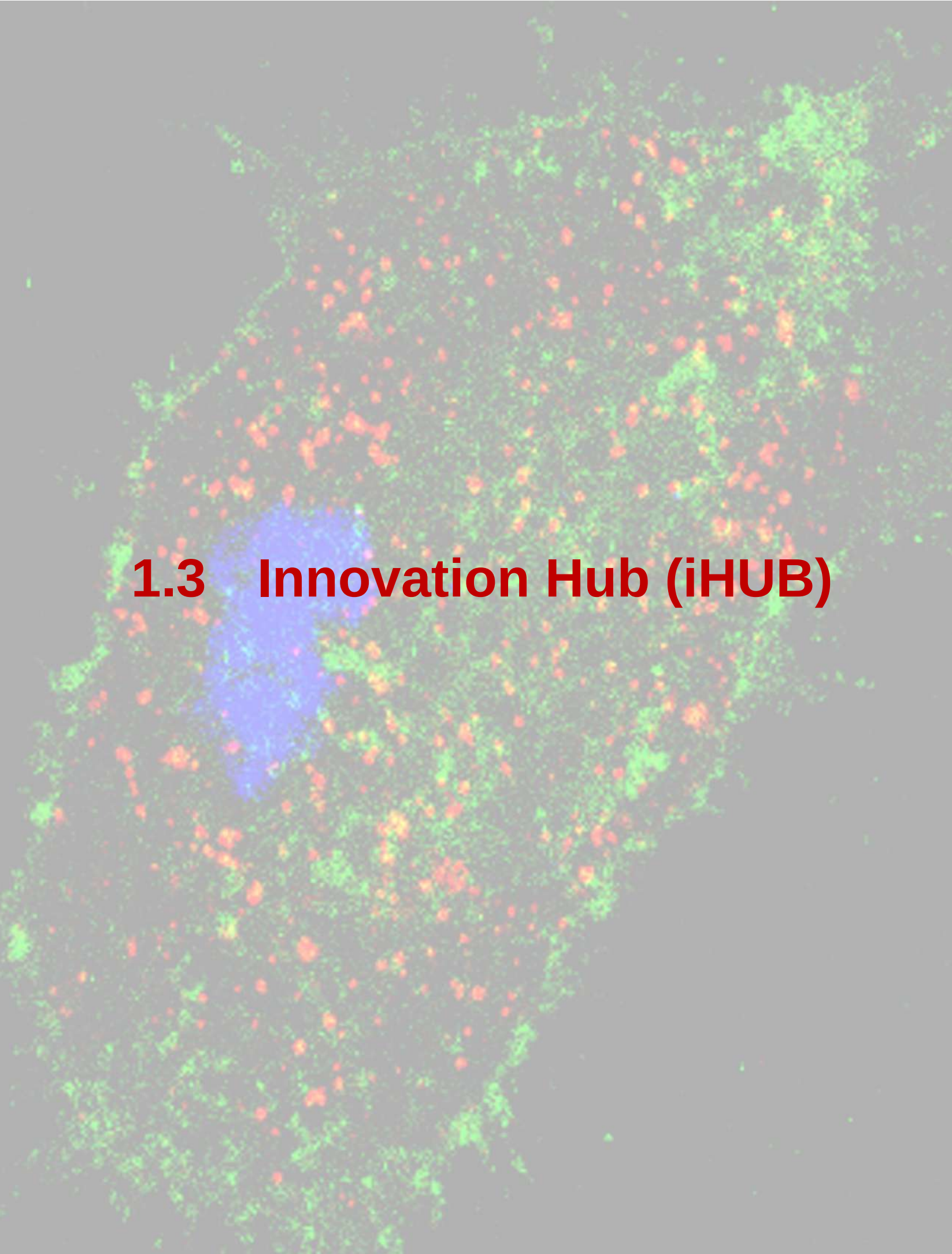
About ten different trainings spanning from 3 days to 4 weeks benefitting around 118 candidates were held - in various advanced areas of CCMB expertise namely, Wildlife Forensics, Cell Biology, CRISPR technology, Microbiology, Recombinant DNA Technology, Proteomics, Stem Cell Biology and Bioinformatics (NGS) during 2018-19. The ultimate aim of these programmes is to improve employability and career advancement (through reskilling/ upskilling) in the area of life sciences. Several entry-level skilling courses in Instrumentation, Laboratory Attendants, Animal Attendants, etc. are in the pipeline as well.

GLIMPSES OF TRAINING PROGRAMMES of

CCMB-SDP

under

CSIR INTEGRATED SKILL INITIATIVE PROGRAMME



1.3 Innovation Hub (iHUB)

1.3. A Services

Consolidated Diagnostics Facility @iHUB

The molecular and chromosomal diagnostic activities have been centralized at the CSIR-CCMB, Annexe-II. The processes have been streamlined from sample collection to report generation. Diagnostic NGS services have been initialized from the month of July. The team has been expanded by new recruitments to cater to the increased demand and for introduction of newer diagnostic tests. An MoU has been inked with CDFD for provision of diagnostic NGS services and to collaborate in areas of common interest.

Molecular Diagnostics

Advances in molecular and cell biology have provided an understanding of the mechanisms of disease at molecular and genetic levels, which can now be translated into diagnostic, prognostic, and therapeutic applications in modern medicine. A number of genetic disorders are known to result from the defects in a single gene. Although rare in comparison to the infectious diseases, genetic disorders cause enormous misery since they are largely incurable and result in many cases, severe morbidity and mortality. In the absence of specific treatments, molecular diagnosis, genetic screening for carrier detection, genetic counseling, pre-pregnancy testing, pre-implantation genetic diagnosis and prenatal diagnosis for these disorders becomes the best approach to prevent their transmission to next generation. The Molecular Diagnostics Facility, CSIR-CCMB, Hyderabad provides diagnostic services for close to 30 such monogenic disorders. The facility provides DNA-based testing for a number of inherited and acquired genetic diseases including hemoglobinopathies, musculopathies, bleeding and clotting disorders and neurodegenerative diseases. The strategy is to identify the causal genetic defect in an individual, screen at risk members for carrier status, tracking inheritance of the genetic defect in the fetus by performing prenatal diagnosis on fetal samples (procured at appropriate stage of pregnancy through hospitals) and providing appropriate and timely genetic counseling. The major thrust of these diagnostic services is to provide reliable genetic testing services to the common man within a rapid turnaround time and at affordable rates.

The advent of Next Generation Sequencing in to clinical practice has tremendously increased out potential to identify the molecular defect in a wide spectrum of genetic diseases. Exome sequencing enables us to screen ~20,000 genes at a go for pathogenic variants. The initiation of NGS diagnostic services is in line with our moto to provide quality, low cost genetic diagnostics to the people of our country and at the same time aid in generation of data important for research and public health care.

Chromosomal Diagnostics

Chromosomal abnormalities are a group of genetic disorders due to microscopically detectable defects at the level of chromosomes. They are commonly implicated in mental retardation, congenital malformations, dysmorphic features, primary and secondary amenorrhea, bad obstetric history, infertility and neoplastic diseases. Cytogenetic evaluation of patients is helpful in the counseling and management affected individuals and families. Prenatal diagnosis of chromosomal abnormalities in high-risk pregnancies helps in detecting chromosomal abnormalities in fetuses and aids in their genetic counseling and reproductive decision making. The state-of-the-art facility offers cytogenetic tests such as karyotyping (conventional-G banding techniques) and FISH (fluorescence in situ hybridization which includes probes using WCP and LSI, mFISH, mBAND, SKY), which involves investigation of genetic defects at the chromosome level.

Wildlife Forensics

At LaCONES, we provide DNA-based species, individual identification, sexing and rehabilitation

services to the nation for the purpose of wildlife crime investigation. We receive biological specimens from all over the country forwarded by forest, judiciary and police department. During the appraisal period (April 01,2018 – March 31,2019) we have received a total 281 wildlife crime cases which includes 554 variety of biological samples such as meat, cooked meat, bones, faeces, dried chemically treated skin, ivory, hair, nails, snake venom, blood stains etc received for identification of animal species. Out of which, 156 cases have been successfully completed DNA forensic analysis and reports were forwarded to the concerned authorities.

During the above mentioned period we have generated revenue of about Rs. 20 lacs from DNA analysis fee.

1.3. B Common Research and Technology Development Hub (CRTDH)

Started in 2014, CRTDH at CSIR-CCMB is one of the few hubs in India that provide technical support, infrastructure and sophisticated analytical services as well advanced research equipment facility to the startups for carrying out competitive technological research. At present, this centre houses 4 companies

- Oncosimis Biotech Pvt. Ltd - involved in a cell engineering to produce biosimilars better than the current benchmark
- Bioartis Life Sciences Pvt. Ltd - developing a point of care device based on DNA amplification for a virus that affects prawn farming
- Virupaksha Life Sciences Pvt. Ltd - optimizing production of biosimilars especially those involved in diabetes
- Kommareddy Bio PharmaPvt. Ltd- repurposing drugs for other clinical contexts
- Althion Tech. Innovations has licensed the water filtration technology from CSIR-IICT to validate the technology towards its application in Type-2 (kidney/hemodialysis purposes) applications. Company is incubating here from August 2018 to optimize the processes in relation to the endotoxin testing and other *in vitro* testing at CRTDH facilities .This aims to facilitiate out licensing the CSIR technologies to private companies for commercial validation and marketability. Pipeline of validation and scale-up will be facilitated by CRTDH to foresee the successful commercialization of the technology. CRTDH's association with research and clinics like Apollo will be helpful to understand the onsite application of such technologies

CRTDH held a Technology Advisory Group (TAG) committee meeting to review applications for incubation received by CSIR-CCMB. Dr Rakesh Mishra views a crucial role of CRTDH in promoting some of the crucial leads by the institute's scientists to translational level. In this context, one of our scientists, Dr Swasti Raychaudhuri's lab has developed a cell based platform to screen drugs for age related diseases like Alzheimer's .The advisory committee strongly recommended his proposal and he will start his activities at CRTDH-CCMB soon.

Prof D. Balasubramanian, Former Director of CSIR-CCMB and Director of Research, LV Prasad Eye and currently advisor to the government of Telangana Institute, gave a talk on the prospects of SMEs/MSMEs in health care sector in India and in particular in Telangana state and how the state government can help them. An interactive session was arranged between the incubating companies and the students and post-doctoral fellows of CSIR-CCMB, which could motivate them towards starting a company with their innovative ideas. A delegation from Nanyang Technological University, Singapore visited our facilities. The discussions were held mainly to see the possibility of helping the startup companies to the best possible extent.

CRTDH–CCMB startups wanted guidance on fundraising for their businesses. We co-organised an interesting and informational session on fund raising through venture capitalists. 50K Ventures, an organization which helps startups get funding gave helpful tips and guidance to the startups.

As the startups at CRTDH–CCMB nearly completed incubation for more than a year, we conducted review of their progress. Periodic review is necessary to ensure that the startup is achieving their milestones as put across in their initial proposal for incubation and avoid digressions. It is also important from the startup's point of view as they can put forth their challenges and problems and seek help from the incubator. The Technology Assessment Group (TAG) committee consisting of CSIR-CCMB scientists and external experts from the biotechnology field reviewed the progress.

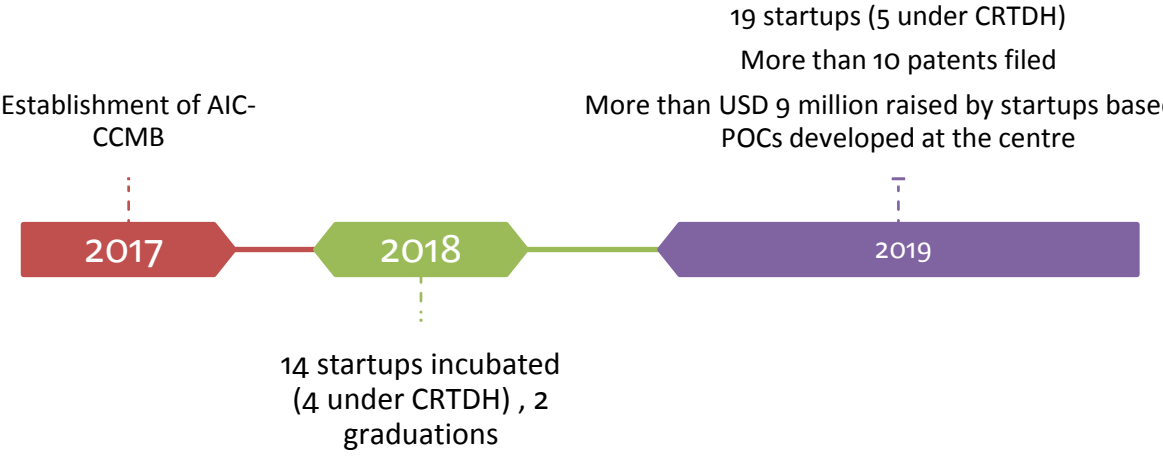
1.3. C Atal Incubation Centre at CSIR-CCMB

CSIR-CCMB is one of the first 10 institutes to establish the Atal Incubation Centre under the Atal Innovation Mission (AIM). Spread over of 10,000 sq. ft space, this centre has one of the largest facilities for shared wet research facilities, industry standard equipment and operating facilities. This centre aims to create an enabling ecosystem of business support services and handholding ecosystem for scientists with early stage technologies and start-ups in health, pharmaceuticals and biotechnology. It is our endeavour to build an ecosystem to enable biotechnology innovation. As Indian researchers and innovators begin to expand the boundaries of science, pursuing novel therapies, diagnostics, medical devices and industrial solutions, we want to ensure that their technologies are translated into sustainable business solutions that reach the citizens.

AIC-CCMB provides customizable lab space to the startups along with industry-standard equipment and facilities for research, workstations, common spaces such as conference, meeting rooms and cafeteria. The start-ups here also have access to ancillary facilities available at the host institute, CSIR-CCMB such as high end equipments, animal house facilities, scientific expertise, library, guest house, etc. Entrepreneurs and scientists with early stage technologies are provided with facilities to develop their proof of concepts.

The past one year has seen AIC-CCMB grow from strength to strength developing a niche for itself in the life sciences innovations space. It has developed partnerships and networks with the key stakeholders such as industry, investors, research hospitals, public funding entities and incubators mostly in Telangana and Andhra Pradesh and also in rest of India. The team was further strengthened with the joining of Dr. Ramjee Pallela as Chief Operating Officer & Ms. Ritika Marampalli as the Manager for Programs and Communications.

As a part of the regular outreach, AIC-CCMB holds regular knowledge sharing sessions called Café Mandala at its premises for the incubatees, students of CSIR-CCMB and the larger life sciences ecosystem. The objective of these meetings is to explore opportunities for business, networking, fund raising and seek support. Each Café Mandala has a specific theme for the meeting depending on what incubatees need information and support on. The centre also holds regular workshops called “Dagar–Pathway to Success. These are held quarterly for business, IP, finance, product development and related topics.



AIC – CCMB celebrated the completion its maiden year in April with an event called 'Pride and Prejudice'. Director General, CSIR, Dr Shekhar Mande, chief guest at the occasion remarked that it is the age of life sciences, and CSIR-CCMB is ideally poised to help the emerging life science start-ups with technical and intellectual expertise. The event was attended by a number of industry doyens like Dr Krishna Ella, Ms Deepanwita Chattopadhyay, Dr A.V. Ramarao, Dr. Satya Prakash Dash and many others. The audience comprised of many prominent scientists, life science industry leaders and enablers to urge the Indian Lifesciences Industry to become partners in the new revolution of home-grown innovations.

Dr. N Madhusudhana Rao, CEO of AIC-CCMB presented the achievements of the centre and showcased its achievements over the last one year. During this period, AIC-CCMB has incubated 8 start-ups working on a range of topics, including diagnostics, food, pharmaceuticals and drug discovery, with two successful graduations. AIC-CCMB has become a prominent player in the larger Telangana startup ecosystem, taking forward the state's thrust on Biotechnology.

I. Magellan Life Sciences secured SOSV Seed fund US$ 225K in Nov.2017 and is presently evaluated at US$ 8.5 mn
II. Theranosis Life Sciences Pvt Ltd secured a US$ 500K from Mumbai Angels and further funding is in pipeline
III. Srikara Biologicals Pvt Ltd, Helixworks Technologies Pvt Ltd and Tardigrade Pvt Ltd have secured GoI and IIT/Pfizer grants


CCMB
Exploratory research
Skill Development
Society
Technical & Analytical Services
Science
Leaders
Innovations
Techinical Expert
Healthcare
Technology Development
Dr. Shekhar C. Mande
Secretary, DSIR
ATAL INCUBATION CENTRE
CENTRE FOR CELLULAR & MOLECULAR BIOLOGY

Startups Incubating at AIC-CCMB

Company Name	Startup Project	Business Remarks
Magellan Life Sciences Pvt. Ltd. *(Graduated October 2018)*	Peptide Based Sugar-free Sweetener	Raised Rs. 1.75 Cr. in 2018 from SOSV. Currently operating from UK with more than 12 global clients including giants like Pepsico.
Helixworks Technologies Pvt. Ltd. *(Graduated October 2018*	Long-chain DNA Synthesis & DNA Coding	BIRAC BIG grantee (Rs. 50 lacs)
Althion Tech Innovations Pvt. Ltd.	Dialysis Grade Water Purification Unit Integrated with IoT	Licensed Make-in-India Technology from CSIR-IICT. Started revenues through orders from Nephroplus
Theranosis Life Sciences Pvt. Ltd.	Microfluidic Chip based Cancer Treatment Monitoring Regime Using AI	BIRAC BIG grantee (Rs. 50 lacs), raised Rs. 3.5 cr from Mumbai Angels
Oncosimis Biotech Pvt. Ltd.	Commercially Important Biologics from ACCET & BACSEC Technologies.	BIRAC BIG grantee (Rs. 50 lacs), which has been done with FDD by BIRAC to fund Rs. 19.5 cr through NBM (results awaited); Pipra anti-counterfeiting awardee in the Bioprocess Engineering Category
Achira Labs Pvt. Ltd.	Fabric Biosensors for Tracking Physiological/ Vital parameters	Matured startup which has already developed two marketable medical devices
BioArtis Life Scienes Pvt. Ltd.	Diagnostic Device for Identifying Infections in Aquaculture	Matured startup recently awarded with NFDB fund of Rs. 1.2 cr
BhapraBiosolutions Pvt. Ltd.	Microencapsulated Mosquito repellent Technology for Military Applications	BIRAC BIG Grantee (Rs. 50 L) and the commercial partners are ready
Sirfbio Pvt. Ltd.	Automated Nucleic Acid Extraction Device	Prototype ready and the team is at the validation stage
Srikara Biologicals Pvt. Ltd.	Poultry Vaccines to Avoid Antibiotics	BIRAC BIG Grantee (Rs. 50 L), currently at the validation stage
Virupaksha Life Sciences Pvt. Ltd.	Pharmacologically Important Biosimilars	Matured firm currently optimized the lab conditions and at the scale-up stage

Bioline Diagnostics (OPC) Pvt. Ltd.	Microtube ELISA Kits for Infectious Diseases and NCDs	PoC completed and the team is at the validation stage
Kommareddy Biopharma Pvt. Ltd.	Drug Repurposing	At the PoC Stage
Laxai Biopharma Pvt. Ltd.	Cost-effective Biosimilars using Novel Recombinant DNA Technology	Matured subsidiary Startup with focused targets in biosimilars
Albot Technologies Pvt. Ltd.	CRISPR Cas Based and Graphene Sensor Integrated Diagnostic Platform	Prospective Disruptive technology in the healthcare area
RR Animal Health Care Pvt. Ltd.	Prebiotic and Probiotic Animal feed for Avoiding Antibiotics	About to finish the PoC
Acrannolife Genomics Pvt. Ltd.	Screening Platform for Panel Reactive Antibodies (PRA) using Artificial Intelligence (AI)	Good traction in the AI ML area and sampling is in pipeline for larger validations
Consytel Life Sciences (P) Ltd.	Promising Anti-Malarial from Plants	Focused Startup with a Co-innovator from CSIR-CCMB
Tardigrade Pvt. Ltd.	Microbial Engineering as Tool to Detect TB and other Infections	At the PoC Stage & Validation
AVRA Life Sciences	Pharmacologically Important Biologics	Early stage Startup with industrial traction
Cube Dx India Pvt. Ltd.	Early Identification of Sepsis Causing Pathogens from Whole Blood using Informatics - ML	Early stage subsidiary Startup in India

2.1 Administration & Management

Research Council

Research Council of a laboratory under CSIR provides direction and vision and helps it to formulate R&D programmes keeping in view the National priorities and opportunity niches and facilitates to design a road map to achieve it. The following are the constituent members of the Research Council of CSIR-CCMB:

Prof G Padmanabhan **Chairman**
Emeritus Professor,
Department of Biochemistry,
India Institute of Science
Bengaluru

Dr R Varadarajan **Member**
Molecular Biophysics Unit
India Institute of Science
Bengaluru

Prof Jitendra P Khurana **Member**
Head, Department of Plant Molecular Biology
Delhi University, South Campus
New Delhi

Prof Umesh Varshney **Member**
Department of Microbiology & Cell Biology
India Institute of Science
Bengaluru

Prof Subrata Sinha **Member**
Director
National Brain Research Centre
Gurgaon

Dr Vijay Chandru **Member**
Chairman & Managing Director
Strand Life Sciences Private Limited
Bengaluru

Dr Krishna Ella **Member**
Chairman & Managing Director
Bharat Biotech International Limited
Hyderabad

Dr Anurag Agrawal **Member**
Director
CSIR-Institute of Genomics and Integrative Biology
Delhi

Dr Samit Chattopadhyay **Member**
Director
CSIR-Indian Institute of Chemical Biology
Kolkata

Dr S Chandrasekhar **Member**
Director
CSIR-Indian Institute of Chemical Technology
Hyderabad

Dr Rakesh K Mishra **Member**
Director
CSIR-Centre for Cellular and Molecular Biology
Hyderabad

Dr K Thangaraj **Secretary**
Chief Scientist
CSIR-Centre for Cellular and Molecular Biology
Hyderabad

Management Council

Following is the composition of the Management Council of CSIR-CCMB for the period 01.01.2018 to 31.12.2019 as approved under Rule-65 of the CSIR Rules 7 Regulations:

Dr Rakesh K Mishra — **Chairman**
Director, CSIR-CCMB
Uppal Road
Hyderabad

Shri S Balaraju — **Member**
Scientist
CSIR-CCMB
Hyderabad

Smt GeethaThanu — **Member**
Senior Scientist
CSIR-CCMB
Hyderabad

Dr Manjula Reddy — **Member**
Chief Scientist
CSIR-CCMB
Hyderabad

Dr Srinivas Voleti — **Member**
Principal Technical Officer
CSIR-CCMB
Hyderabad

Dr S Chandrasekhar — **Member**
Director
CSIR-Indian Institute of Chemical Technology
Hyderabad

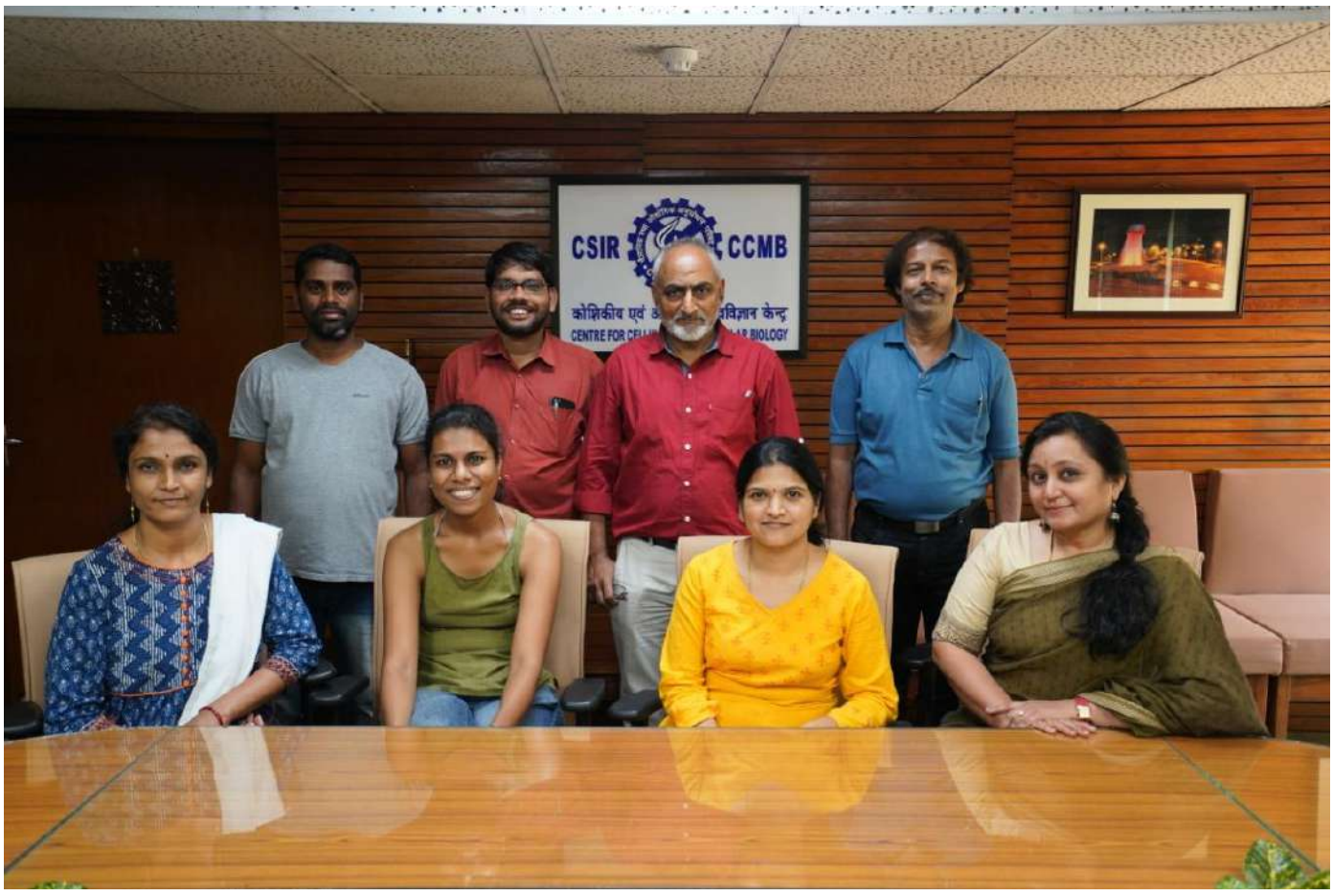
Dr Archana B Siva — **Member**
Principal Scientist & Head, Business Development
CSIR-CCMB
Hyderabad

Dr Shashi Singh — **Member**
Chief Scientist
CSIR-CCMB
Hyderabad

Finance & Accounts Officer — **Member**
CSIR-CCMB
Hyderabad

Controller of Administration — **Member-Secretary**
CSIR-CCMB
Hyderabad

Director’s Office



Administration

The overall administration of the Centre and the supervision of ancillary services such as transport and telecommunications are under the purview of the administration. In addition, secretarial assistance is provided to the staff for the preparation of the reports, manuscripts and correspondence.

Finance & Accounts

All financial matters pertaining to CSIR-CCMB, including budget planning, allocation and expenditure are taken care of by the Finance and Accounts section.

Stores & Purchase

CSIR-CCMB has a modern stores building with a cold storage facility and separate rooms for the storage of solvents and acids. The Stores and Purchase section maintains an exhaustive inventory of inorganic chemicals, stationery, glassware, plastic ware and other items. The staff of this section carries out the processing of orders and the procurement of materials for the Centre.

Security

The Security services are outsourced to a professional security agency and is under supervision of trained security officers of CSIR-CCMB.

Medical Services

CSIR-CCMB shares a well-equipped clinic and dispensary with the CSIR-IICT. Medical care is available round the clock for staff and their families.

Guest House Staff

CSIR-CCMB's guest house has 28 well-furnished suites/ rooms and accommodates visitors from India and abroad.

Canteen Services

CCMB Canteen has three restaurants that serve all catering needs of the staff and is functional on all working days.

Planning Monitoring and Evaluation Group

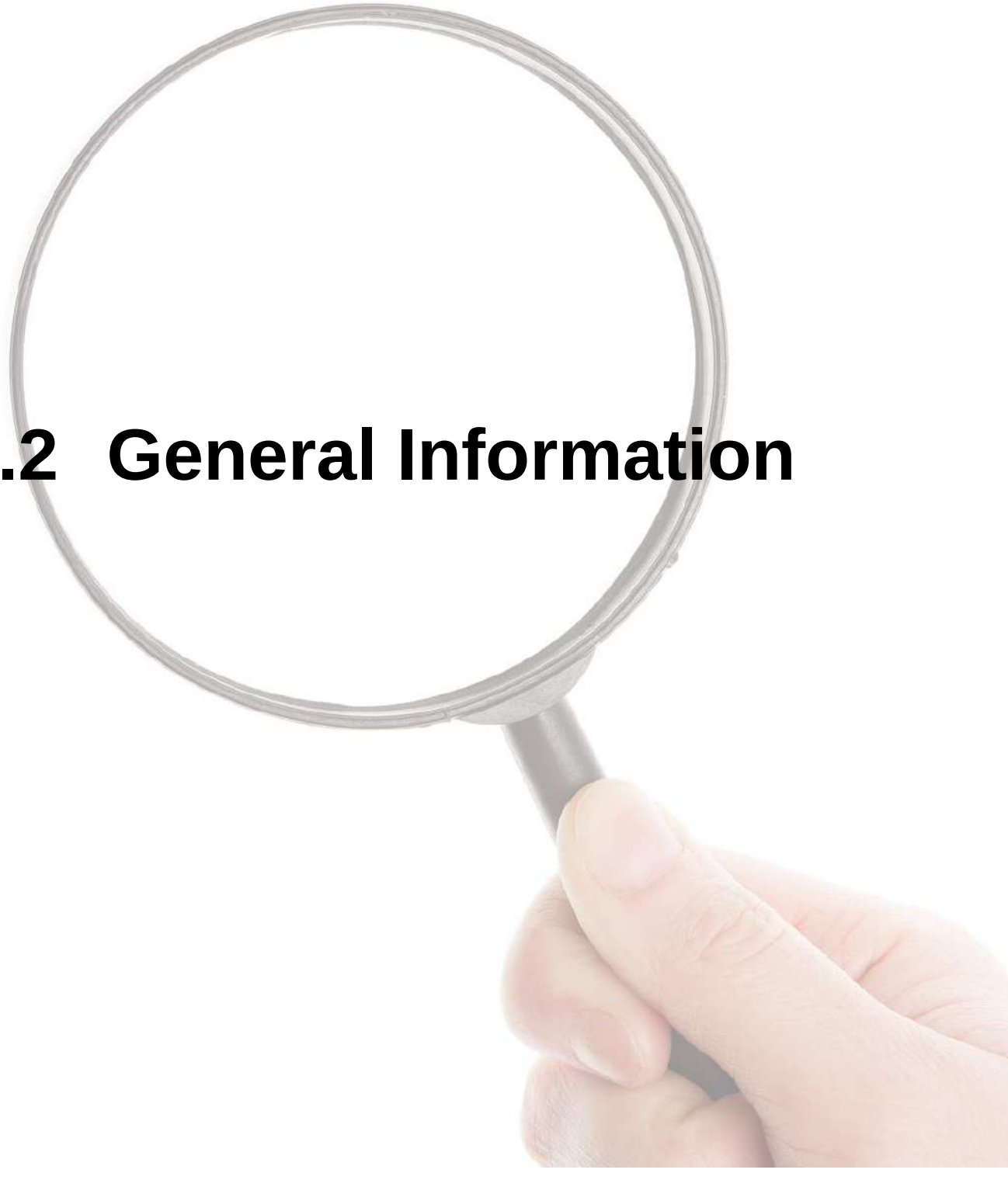
The primary responsibility is to assist the Director, CSIR-CCMB in project management activities and act as a liaison between the Director and other research groups, CSIR-HQ and other organizations. The PME takes care of various in-house, sponsor, collaborative, grant-in-aid and NIMTLI projects and provides inputs related to projects. In addition, PME provides information to project audit agencies and RTI queries.

PME assists the Director in preparation and collating institutional data for onward transmission to CSIR headquarters, survey agencies. PME also conducts various institutional programs as advised by the Director from time to time.

Business Development Group

Business Development Group of CCMB carries out various activities related to technical services, IPs and technology transfers. Technical services include diagnostics services (Molecular Diagnostics, Wildlife Forensics & Chromosomal Diagnostics) and various analytical services. BDG coordinates with CSIR HQ for facilitating the research leads from CCMB for patenting. The group also facilitates CCMBs connect with industry for contract & collaborative research projects, technical services, tech transfers, trainings, etc.

2.2 General Information



2.2. A List of Publications

1. Abraham A., Thirumalairaj K., Gaikwad N., Muthukkaruppan V., Reddy A., Thangaraj K., Kim U., Vanniarajan A. (2019) Retinoblastoma discordance in families with twins. Indian Journal of Ophthalmology. 67(3): 436-439.
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2.2. B Awards & Honors

Research Staff

K Thangaraj

- Dr. Joseph Thomas Memorial Lecture Award, IIT Madras, Chennai
- International Centre for Genomic Medicine in Neuromuscular Diseases, funded by MRC, UK (Other partners: UK, Brazil, South Africa, Zambia & Turkey)
- Prof. C. V. Pichappa Endowment Lecture Award, Department of Biochemistry, University of Madras, Chennai
- Prof. G. Jayaraman Endowment Lecture Award, Department of Genetics, University of Madras, Chennai
- Associate Editor for Tropical Medicine and International Health, Wiley Press
- Sun Pharma Research Award for the year 2018 in the field of 'Medical Sciences - Basic Research'
- Elected Fellow, National Academy Sciences – India, Allahabad
- Dr. Palle Rama Rao Award, by Government of Andhra Pradesh, at Andhra Pradesh Science Congress, Kadapa

G R Chandak

- Fellow at National Academy of Medical Sciences, New Delhi, India
- Prof. I C Verma Oration from Indian Society of Human Genetics
- Member, Advisory Committee, Mission program on Genomics and Computational Biology, DBT, New Delhi
- Member, Technical Advisory Committee, Indian Statistical Institute
- Dr Prabha Mehra Foundation Day Oration on the 88th Foundation Day of Dept of Obs & Gyn, KGMU, Lucknow
- Member, Technical Review Committee, SERB, DST
- Expert Member on the 'Chronic Disease Biology' TEC, DBT
- Member, SAC, National Institute of Biomedical Genomics, Kalyani
- Delivered SS Agarwal Memorial Lecture, SGPGIMS, Lucknow
- Member, Expert Committee for evaluation and review of Multi-Centric Network project on 'Indian Human Microbiome Initiative'
- Member, PAC on Health Sciences, SERB

Manjula Reddy

- Member, Editorial Board, Journal of Bacteriology

Amitabha Chattopadhyay

- Awarded SERB Distinguished Fellowship
- Elected as Fellow of TWAS
- Invited to be Distinguished Visiting Professor, IIT, Bombay
- Invited to be Adjunct Professor, IISER, Kolkata
- Invited to be a Lead Speaker at the Faraday Discussion on Photo-induced Processes in Nucleic Acids and Proteins, Kerala
- Elected as Member, Board of Studies of Biological Sciences, Academy of Scientific and Innovative Research
- Awarded 6th G.K. Manna Memorial Lecture Award

Yogendra Sharma

- Invited to be Adjunct Professor, IISER, Berhampur
- Elected Fellow, Indian National Science Academy
- Awarded J C Bose Fellowship

Anant Bahadur Patel

- EK Zavoisky Fellowship from International Society for Magnetic Resonance in Medicine (ISMRM) to attend the joint Annual Meeting of ISMRM-ESMRMB, Paris, June 2018
- Two oral presentations and posters in the joint Annual Meeting of ISMRMESMRMB, Paris, June 2018

Jyotsna Dhawan

- Invited speaker, FASEB Summer Research Conference on Muscle Satellite and Stem Cells, Keystone USA
- Chair, Raman Research Institute Trust
- President, Indian Society of Developmental Biology

Deepa Selvi Rani

- The Servier Young Researcher Award by WINCARS Association, Bangalore

STUDENTS

D V S Sudhakar

- Travel fellowship from European Society of Human Genetics (ESHG) for attending ESHG-2017 held at Copenhagen, Denmark
- Developing Country Travel Award to attend the American Society of Human Genetics Annual Meeting in San Diego, California, USA
- K. V. George Kottukulam Memorial TANKER & Kerala Kidney Research Foundation Young Investigator Award

Sunil Tripathi

- Travel fellowship from World Leish Organization to attend the 6th World Congress on Leishmaniasis held at Toledo, Spain

Nipa Basak

- Newton-Bhabha PhD Placements Award by the Department of Science & Technology and British Council to visit King's College, London, UK
- Best poster award at the 4th International Congress on Epigenetics & Chromatin" held during September 03-05, 2018 in London, UK
- Full bursary for "WGC Advanced Course: Next Generation Sequencing Bioinformatics" during October 07-13, 2018 at Wellcome Genome Campus, Hinxton, UK

Sneha Paturi

- Best Poster award 24th National Magnetic Resonance Society Meeting held at IISER, Mohali

Satya Brata Routh

- Indian National Science Academy - INSA MEDAL for Young Scientists 2018

Sreetama Pal

- Best Poster Award at the international conference "Recent Advances in Molecular Spectroscopy : Fundamentals & Applications in Materials and Biology" University of Hyderabad

Satyajeet Salunke

- Best Poster presentation at the XLII All India Cell Biology Conference of Indian Society of Cell Biology, held at BITS Pilani, K.K.Birla Goa campus, Dec, 2018

Mazeed Mohd

- Best Poster Award at the 24th Congress and General Assembly of the International Union of Crystallography, IUCr-2017, held at Hyderabad International Convention Centre

Komal Ishwar Pawar

- Best Poster Award at the 11th AARS IUBMB Focused Meeting on Aminoacyl tRNA Synthetases, held at Florida, USA

Santosh Kumar Kuncha

- Best Oral Presentation at 9th RNA group meeting held at BHU, Varanasi
- DST-DFG Award for participation in the 68th Meeting of Nobel Laureates, Lindau, Germany

Suraj S Nongmaithem

- Award for best Short Talk from Indian Society of Human Genetics
- Dr S S Agarwal Young Scientist Award from Society of Indian Association of Medical Genetics

Meraj Ahmad

- Award for best Short Talk from Indian Society of Human Genetics

Lovejeet Kaur

- Abstract accepted for oral presentation at the 5th International Vitamin Conference 2018, Sydney

Shanti Swaroop Srivastava

- Ratna Phadke Award of Indian Biophysical Society (IBS), Annual Meeting at IISER, Mohali

Selected as Fulbright-Nehru Fellow to work in USA (August 2018)

Radhika Khandelwal

- Carl Storm International Diversity (CSID) fellowship from Gordon Research Conference (GRC) to attend GRC-Calcium Signaling Conference at Renaissance Tuscany Il Ciocco Lucca (Barga), Italy.
- DST-DAAD visitor (Nov 2018) to the University of Oldenburg, Germany

Pratibha Bhalla

Travel grant for "15th Asian Conference on Transcription" held at Penang, Malaysia

Sadiya Parveen

Young Scientist Award for the year 2017 by Telangana Academy of Sciences

Shagufta Khan

- Selection for poster presentation entitled "Transgenerational Epigenetic Inheritance via the Male Germ Line in Drosophila melanogaster" at Epigenetics and Chromatin meeting going to be held at Cold Spring Harbor Laboratory, New York, USA
- Travel award of ICMR, DBT and CSIR to present her work in Epigenetics and Chromatin meeting going to be held at Cold Spring Harbor Laboratory, New York, USA (11th – 15th Sept 2018)

Fathima Athar

- Presentation of poster entitled "Simple Sequence Repeats (SSRs) – Guardians of the Chromatin Galaxy" at the Annual Meeting of International Society for Stem Cell Research (ISSCR) held in Melbourne, Australia

Rahul Sureka

- Presentation of poster entitled "Evolutionary Conservation of Nuclear Matrix Proteins" at EMBO Conference Series on Nuclear Structure and Dynamics held in L'Isle sur la Sorgue, France

Gopal Kushawah

- Presentation of poster entitled "Functional Analysis of Extremely Conserved Non-Coding Sequences in Zebrafish" at the EMBO – EMBL Symposia: The Non-Coding Genome held in EMBL Heidelberg, Germany

Divya Tej Sowpati

Oral presentation entitled "C-State: An Interactive Epigenome Browser" at the European Conference on Computational Biology (ECCB) held in Prague, Czech Republic

Anand Sharma

DST-DAAD visitor (Nov 2018) to the University of Oldenburg, Germany

Ajoy Aloysius

- Platform Presentation award at ICCB Jan 2018 on "Quietly changing partners: Smad3 replaces β-catenin in Tcf/Lef transcriptional activation in quiescent muscle stem cells"
- DST-SERB Travel award to present a poster at the European Wnt Meeting in Heidelberg, Germany (Sept 2018)

Swetha Sundar

- Travel grant by the organizers to attend Advances in Stem Cell Biology course in April 2018 at Institut Pasteur, Paris

Lamuk Zaveri

- ISSCR Travel Award to attend International Society of Stem Cell Research annual meeting in Australia, June 2018, to present a poster “Oct4 and KLf synergize to target cell cycle regulators during reprogramming”

Asmita Pawar

- Awarded EMBO Travel Grant for attending ‘EMBO mPEPC1’ course at EMBL, Germany (Sept 2018)

Ashish Jha

- Scholarship to attend Advanced Field course in Ecology & Conservation at Xishuangbanna Tropical Botanical Garden Chinese Academy of Sciences, Yunnan Province, China

Debarya Saha

- EMBL Travel award to attend the EMBL Conference on Transcription and Chromatin in Heidelberg, Germany 2018

Parijat Sarkar

- Sun Pharma Science Scholar Awards-2018 in Biomedical Sciences and Pharmaceutical Sciences

Sujoy Deb

- Best Poster Award at the International conference on Genome Architecture and Cell Fate Regulation held at HCU, Hyderabad, Dec, 2018

A Paramasivam

- Travel award from the American Society of Human Genetics (ASHG) to attend ASHG Conference 2018, San Diego, USA
- Travel award from the European Society of Human Genetics (ESHG) to attend ESHG Conference 2018, Milan, Italy

Chhavi Dawar

- Awarded GYAN scholarship to attend 8th International Nextgen Genomics, Biology, Bioinformatics and Technologies Conference (NGBT 2018), Jaipur India.
- Received "Nature Genetics Best Poster Prize"

S Manu

- National Geographic Early Career Grant (NatGeo Young Explorer Award) US $5000 for covering field expenses

Parna Saha

- Travel award of SERB to present her work at EMBL Conference: Transcription and Chromatin, EMBL Heidelberg, Germany (2018)

Avinash Srivastava

- Travel award of DBT, ICMR to present his work at EMBL Conference: Transcription and Chromatin, EMBL Heidelberg, Germany, 2018

Sohini Deb

- Best Poster Award at the 59th Annual Conference of Association of Microbiologists of India & International symposium on Host-Pathogen Interactions at University of Hyderabad

Pankaj Kumar

- Received Gordon Research Conferences’ GRC Carl Storm International Diversity
- (CSID) Award and the ICMR travel Grant to attend the Gordon Research Conferences on Drug Resistance held at Bryant University in Smithfield, RI, USA

Prachand Issarapu

- Young Scientist Award by Indian Society for Human Genetics

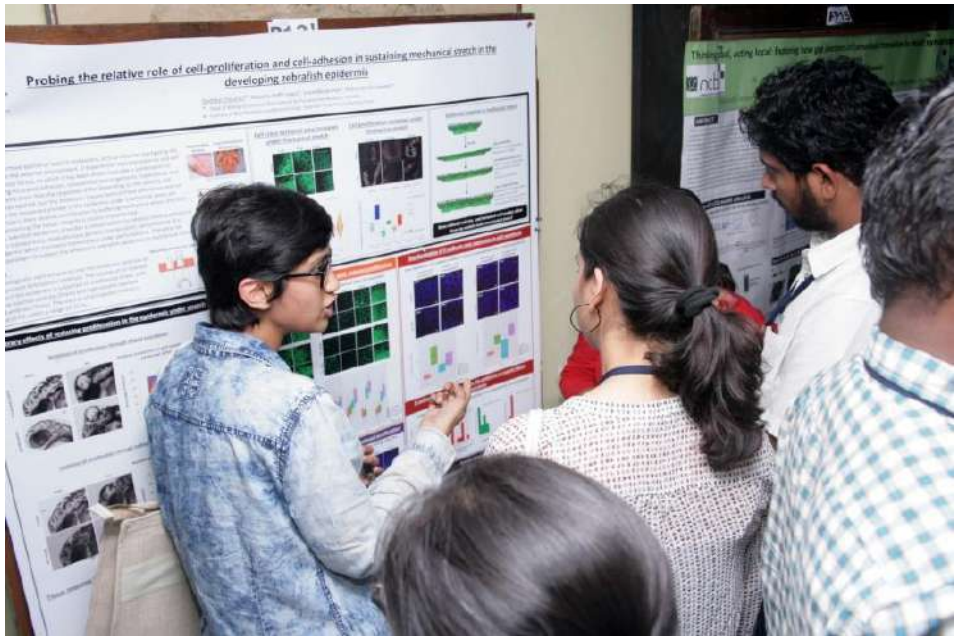
2.2. C Conferences & Symposia

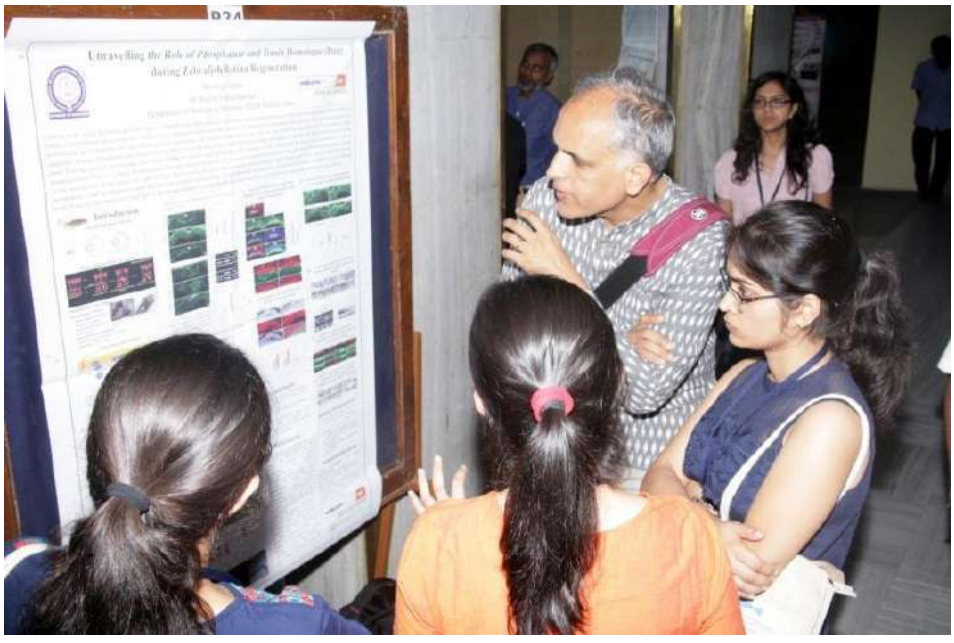
Workshop on Introduction to Modelling Species Distributions

CCMB-LaCONES organized a workshop titled, “An Introduction to Modelling Species Distributions” in its campus from June 25 - 29, 2018. It was conducted in collaboration between Colorado State University and CCMB, as a part of US –India 21st Century Knowledge Initiative programme by United States – India Educational Foundation (USIEF).

iZIM 2018

Indian Zebrafish Investigators Meeting (iZIM) is a biennial meeting held aiming to bring the growing number of zebrafish researchers in India together to form a community. 76 participants including 26 Faculty from various research institutes and universities in the country participated in iZIM2018 hosted by the CSIR-CCMB from July 3-6, 2018.





Workshop on Communicating Conservation Research

A workshop on COMMUNICATING CONSERVATION RESEARCH was held at LaCONES (CSIR-CCMB) Hyderabad during July 30-August 3, 2018. This workshop was organized by LaCONES (CSIR-CCMB). Dr. Asad Rahmani, former Director of Bombay Natural History Society and former editor of Journal of Bombay Natural History Society was the resource person and designed the workshop curriculum. Thirteen students of ecology from various parts of India, working on different aspects and taxa were selected for this workshop.

On August 2, 2018, Dr Rahmani gave a popular talk on Migration of Birds at Goethe-Zentrum, Banjara Hills. This public talk was organized by LaCONES, in association with BNHS and Goethe-Zentrum (German Center).

Dedication of National Wildlife Genetic Resource Bank to the Nation and Reintroduction of Indian Mouse Deer into the Wild

The Hon'ble Minister for Science & Technology, Earth Sciences and Environment, Forests and Climate Change, Dr.HarshVardhan, dedicated to the Nation the National Wildlife Genetic Resource Bank at CCMB-LaCONES on August 12, 2018. The Wildlife Genetic Resource Bank at LaCONES can store 17,000 vials worth of samples. The bank currently stores genetic material, including DNA, cells and tissues besides semen and eggs of 23 wildlife species.

Also the first batch of Indian Mouse Deer, as part of the first ever planned reintroduction of the species at Farahabad range of Amrabad Tiger reserve, was flagged off by the Hon'ble Minister from Nehru Zoological Park, Hyderabad.

HyTiCoS and CCMB Talk

HyTiCoS and CCMB jointly hosted talk on **Ornithology: Past, present and future** by Bikram Grewal and

Reinventing Wildlife Conservation by Bittu Sahgal on September 22, 2018, in CCMB-LaCONES.

Pre-IISF event

The Pre-IISF event, a precursor to the 4th India International Science Festival (IISF) to be held in Lucknow from October 5-8, 2018, was held as part of the Open Day programme on September 26, 2018.

The students and visitors were encouraged to write any science related question and a seal of "Curious Scientist" was put on their hand as an appreciation. Therewere many interesting questions asked and CCMB team will pick a few of them and provide answers on its blog.

2nd National PostDoc Symposium 2018

The 2nd National Post Doc Symposium was a joint endeavor by NCBS, InStem and CCMB PostDoctoral fellows to bring together Life Science PostDocs across the country. 70 Post Docs from across the country attended this 3-day symposium comprising of invited talks, research presentations, mentor interactions, informative panel discussions and workshops hosted by CSIR-CCMB from October 3-5, 2018.

Wiley Author Workshop

The publishing house, Wiley organised an Author workshop with CCMB PhD students, postdocs and scientists on October 24, 2018. The short workshop aimed at discussing how to write academic papers for an easier publishing experience. They covered various relevant topics in the session such as choice of an appropriate journal, importance of writing papers concisely but without compromising the details that can affect reproducibility of thr works, possibilities of plagiarism that can come up, rebuttal procedures and resubmissions. The workshop also realised need for lowering publishing costs for open access journals in an Indian context.

Causins Meet-IV

As part of the CSIR-AIST United Symposium on Innovative and Industrial Sciences, a one-day event was held in CCMB with Scientific talks by faculty from the DAICENTER, BMRI, Japan and the CSIR-CCMB on November 22, 2018.

Director, CCMB with Former British PM Mr. David Cameroon

On July 19, 2018, Director, CCMB, was part of the delegation which met Former British PM Mr David Cameroon to explore the opportunities that await India through integration of genomics and genetic medicine into medical practice and acceleration of this medical revolution for all Indians.

2.2. E MoUs & Agreements

Research MOU

- Sickle Cell Institute Chhattisgarh, Raipur
- Government Medical College, Nagpur, Maharashtra
- Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad

Academic MOU

- Academic Exchange Program with IIT, Bombay
- Academic Exchange Program with IIT, Kharagpur
- Academic Exchange Program with NIPER, Guwahati
- Academic Exchange Program with0020IIT, Hyderabad

Institutional MOU

- Acharya N.G. Ranga Agricultural University, Guntur
- Telangana State Forest College & Research Institute, Hyderabad

CCMB-Atal Incubation Centre and CRTDH Agreements

- National Biodiversity Authority
- RR Animal health Care Limited, Hyderabad
- Srikara Biologicals Pvt. Ltd., Tirupati

Exchange of MoU with APCARL

CCMB entered into an MoU with APCARL. CCMB will provide technical inputs and support for functionalizing the Animal House and Biosafety labs at APCARL; setting up a Skill Development Program and a Food Safety Laboratory.

2.2. F Visitors & Invited Talks

Spanish Delegation

Adrian Gutiérrez, Counsellor for Science and Technology at theEmbassy of Spain visited CCMB on October 05, 2018. Meeting was to explore collaborative opportunities in research.

Visit of Junior Judges to CCMB

- Junior Judges from the Judicial Academy visited on July 16; September 24 and December 05 of 2018.
- Dr. K Thangaraj delivered lecture on “DNA Fingerprinting”

140 IFS Officers visited CCMB-LaCONES on October 05, 2018

Visit of Dr. Uma Valeti, MD, CEO & Co-Founder, Memphis Meat, California on 15th June, 2018

Visit of Labour Commissioner, Government of India, Southern Region, Hyderabad

2.2 G Invited Talks

Dr Abhishek Asthana

Case Western Reserve University, Cleveland, USA

"Acute Myeloid Leukemia therapy: a novel target"

10 April 2018

Padma Shri Prof D. Balasubramanian

(An AIC-CCMB Event)

"Opportunities and Imperatives: Biotech in India"

16 April 2018

Dr Indrajit Lahiri

University of California, San Diego, USA

"Unraveling fundamental nucleic acids processes: the role of cryo – EM"

17 April 2018

Dr Selvi Bharathavikru

MRC Human Genetics Unit, Western General Hospital, Edinburgh, UK

"RNA regulation in development and disease; insights from Wilms Tumour 1"

01 May 2018

Dr Anil Koul

Director IMTECH, Chandigarh

"Bacterial and viral lung infections and new emerging therapies"

11 May 2018

Dr Manoj B. Menon

Institute of Cell Biochemistry, Hannover Medical School, Germany

"Cell type specificity of septin cytoskeleton what we learnt from the Sept7 knockout model"

28 May 2018

Dr Sudarshana M Sharma

Hollings Cancer Center, Medical University of South Carolina, Charleston, USA

"Genomics to transgenics: Understanding gene regulatory networks involved in bone destruction"

12 June 2018

Dr. Souvik Modi

Department of Neuroscience, Physiology and Pharmacology,

University College London, UK

"Designer nanoprobes to investigate cellular functions"

20 June 2018

Mr. Lakshman Sundaram

Stanford University, California, USA

"PrimateAI - Predicting the clinical impact of human mutation with deep neural networks"

03 July 2018

Prof. Animesh Ray

Keck Graduate Institute, Claremont, USA

"Noncoding RNA in melanoma: rheostats and integrators"

09 July 2018

Dr Bhavana Muralidharan

UK Dementia Research Institute, UCL, London

"Building the brain: Role of Transcription factors and chromatin regulators"

13 July 2018

Dr Ramakrishna Chandran

City of Hope Beckman Research Institute, California, USA

"Gut microbiota and Brain Function: Studies in mouse models of Viral encephalitis and chemotherapy induced neuropathic pain"

16 July 2018

Dr Chiranjit Chowdhury

Dept. of Biochemistry, Iowa State University, USA

"Towards understanding substrate transport across microcompartment shell"

23 July 2018

Dr Quasar S Padiath

Dept. of Human Genetics, Graduate School of Public Health,University of Pittsburgh, USA

"The nuclear lamina and the regulation of cellular function, development and disease"

08 August 2018

Dr Bivash Pandav

Scientist and Wildlife Biologist, Wildlife Institute of India

"Saving tigers in a human dominated landscape - the Terai experience"

17 August 2018

Dr Krishnan Raghunathan

Research Assistant Professor, University of Pittsburgh,USA

"How do bacterial toxins associate with membrane rafts?"

20 August 2018

Dr Arjumand Ghazi

University of Pittsburgh School of Medicine, USA

"Fat, Fertility and Immunity in an Aging Worm"

21 August 2018

Dr Devram Ghorpade

Department of Medicine,Columbia University, New York, USA

"Obesity: Liver dictates meta-inflammation via secretory hepatokine, dipeptidyl peptidase 4 (DPP4)"

23 August 2018

Prof Petra Levin

Department of Biology, Washington University at St Louis, USA

"Metabolism, Microbes and Mice: the impact of the environment on bacterial growth and physiology"

10 September 2018

Prof Abhijit Chakrabarti

Saha Institute of Nuclear Physics, Kolkata and Homi National Institute, Mumbai.

"The Chaperone Story of Spectrin"

18 September 2018

Dr Mridula Nambiar

Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, USA

"Role of cohesins in regulating centromeric recombination and segregation during meiosis"

20 September 2018

Prof Krishnananda Chattopadhyay

Structural Biology and Bioinformatics, CSIR-Indian Institute of Chemical Biology, Kolkata

"Protein conformation, dynamics and aggregation: in a test tube, on surface, and in human diseases"

28 September 2018

Dr. Meena Venkataraman

Wildlife Biologist, Carnivore Conservation & Research (CCR) Mumbai

"Asiatic lion research from coalition to conflict"

08 October 2018

Prof. Girjesh Govil

TIFR, Mumbai

"NMR and X-rays in Chemistry, Industries and Biology"

10 October 2018

Dr Shravanti Rampalli

InStem, Bangalore

"Expanding the Roles of Histone Lysine Methyltransferases Beyond Gene Regulation in Reprogramming and Aging"

15 October 2018

Dr G V Ramanjaneyulu

Executive Director, Centre for Sustainable Agriculture, Hyderabad

"Agrarian distress and emerging alternatives"

16 October 2018

Dr. Naveen Kumar Navani

Associate Professor, Department of Biotechnology, IIT Roorkee

"Bioengineering Approaches for Selection of Synthetic Functional Nucleic Acids"

26 October 2018

Dr. Jahnavi Joshi

Royal Society-SERB Newton International Fellow, The Natural History Museum, London, UK

"Systematics, biogeography, and diversification in Asian tropical forests: case studies from centipedes and butterflies"

02 November 2018

Dr Raushan K Singh

University of Massachusetts Medical School, USA

"SWR1C: A nucleosome editing machine"

12 November 2018

Dr. Abhiram Dukkipati

Magellen Life Sciences, Hyderabad

"Signal Transduction by Sweet Proteins: From Structural Biology to a Global Product"

15 November 2018

Dr Suresh Kumar

Department of Molecular Genetics & Microbiology, University of New Mexico, Albuquerque, USA "Stx17: from a SNARE to a master regulator of autophagy"

21 December 2018

Dr Vivek Chopra

Senior Scientist, Translational development in oncology, Amgen inc. San Francisco, CA

21 December 2018

Dr Vinod Scaria

IGIB, New Delhi

"Personal Genomes to Precision Medicine"

04 January 2019

Dr Binay Panda

Ganit Labs Foundation, Bangalore

"Data Integration in Cancer Biology and why it is important to find clinically relevant signatures"

04 January 2019

Dr Antony M Jose

University of Maryland, USA

"How life preserves form and function across generations"

07 January 2019

Dr. Rajeev Kumar

INRA, Versailles, France

"Antagonistic roles of BRCA2 and the FIGL1 complex regulate homologous recombination repair during meiosis"

08 January 2019

Dr Meghna Krishnadas

Yale School of Forestry and Environmental Studies, USA

"Edge effects in fragmented forest erode mechanisms that maintain plant diversity"

08 January 2019

Dr. Kousik Kundu

Department of Human Genetics, Wellcome Sanger Institute, Hinxton, UK

"Genetic and genomic approaches to understand human complex diseases"

09 January 2019

Dr Wasimuddin

Institute for Infectious Diseases, University of Bern, Bern, Switzerland

"Host-parasite, host-microbiome interactions: insights from natural systems"

09 January 2019

Dr. Purushothama Rao Tata

Department of Cell Biology, Duke University School of Medicine, Durham, USA

"Plasticity in tissue regeneration and tumorigenesis"

10 January 2019

Dr Sanchari Bhattacharyya

Department of Chemistry & Chemical Biology

Harvard University, Cambridge, MA, USA

"A biophysical approach to understand molecular driving forces of evolution"

10 January 2019

Dr. Jyoti Shah

University Distinguished Research Professor,BioDiscovery Institute, University of North Texas, "Actin dynamics involving the Actin depolymerizing factor 3 and plant defense against insects that feed from the phloem"

17 January 2019

Dr Ashsih Ranjan

Department of Plant Pathology, University of Wisconsin - Madison, USA

"Deciphering soybean resistance mechanism to Sclerotinia stem rot"

28 January 2019

Dr Appu K Singh

Department of Biochemistry and Molecular Biophysics, Columbia University, New York, USA

“Structure and Function of Epithelial Calcium Channel TRPV6”

07 February 2019

Dr Amitabha Mukhopadhyay

Kusuma School of Biological Science, Indian Institute of Technology, Delhi.

“Salmonella effectors mimic the host Syntaxins to avoid lysosomal targeting”

07 February 2019

Dr Kundan Sengupta

IISER, Pune

“Divided we stand, united we fall" - a saga of the nucleolus

08 February 2019

Dr Budhaditya Mukherjee

University of Geneva, Switzerland

“Dissection of the Fundamental Roles Played by Apicomplexan Aspartyl Proteases in Establishment of Parasitism”

13 February 2019

Dr Mohan R. Wani,

National Centre for Cell Science, Pune

“T lymphocyte derived molecules regulate biology and pathophysiology of bone cells”

15 February 2019

Professor Dr. Karl-Wilhem Koch

Dept. of Neuroscience, University of Oldenburg, Germany

“Second messenger homeostasis in rod and cone cells”

15 February 2019

Dr Arnab Barik

National Institutes of Health, Bethesda, USA

“Brainstem Control of Response to Pain and Itch”

20 February 2019

Dr Anirban Basu

National Brain Research Centre, Gurgaon

“Drug repositioning/repurposing: Promising strategy to develop therapy against viral infections”

27 February 2019

Dr Deo Prakash Pandey

Dept. of Microbiology, Rikshospitalet

“Identification and characterization of novel epigenetic regulators of gliomas”

01 March 2019

Dr. Poonam Thakur

Institute for Neurophysiology, Goethe University, Frankfurt, Germany

“Parkinson`s disease - Harnessing the animal models to understand the pathophysiology”

05 March 2019

Prof David Roper

University of Warwick, UK

“The relationship of bacterial cell wall synthesis to cell division and antibiotic resistance”

11 March 2019

Dr Ashutosh Kumar

Department of Biosciences and Bioengineering, IIT Bombay, Mumbai

“Disordered tails tune the order inspecialized nucleosome formation”

18 March 2019

Dr Ishan Agarwal

Post Doctoral Fellow, NCBS, Bangalore

“India as a model region for studies in biogeography and diversification”

21/Mar/2019

2.2. H CCMB Events & Popular Talks

Independence Day (August 2018)

On August 15, 2018, Dr. Rakesh Mishra, Director, CCMB, hoisted the flag and distributed prizes and certificates to the winners of various indoor and outdoor sports conducted by the Staff Club.

Hindi Week (September 2018)

The Hindi Day celebrations were conducted on September 14, 2018 by Prof. Arun Tiwari.

CCMB Foundation Day (November, 2018)

The CSIR-CCMB laboratory complex in the campus at Habsiguda was officially dedicated to the nation and cause of science in the year 1987 on November 26. CSIR-CCMB continues to celebrate this day every year as its Foundation Day. The day is marked by a student symposium in the forenoon followed by a lecture by an invited Speaker, a distinguished scientist of international repute.

The Speakers for the student symposium were Gajanan Patil, ParijatSarkar, Shivali Rawat, Zuberwasim Sayyad, Shagufta Khan, Pavan Kumar, Sohini Deb, Divya Sriram and Debarya Saha. At the end of the symposium, the Anindya Kumar Ghosh memorial award for 2017 batch was awarded to Mansi Srivastava and Bedaballi Dey.

Padma Shri Dr K Radhakrishnan, Former Chairman, Space Commission/ Secretary, Department of Space & Chairman, ISRO, currently Honorary Distinguished Advisor, Department of Space/ ISRO, Antariksh Bhavan, Bengaluru, and Advisor, delivered the Foundation Day lecture on India and the New Space Age.

Republic Day (January, 2019)

On January 26, 2019, CCMB invited Justice Neelam Sanjiva Reddy, Former Judge of High Court of Andhra Pradesh, as the Chief Guest. Dr. Rakesh Mishra, Director, CCMB, hoisted the flag followed by a speech by Justice Neelam Sanjiva Reddy. Prizes and certificates were distributed to the winners of various indoor and outdoor games.

Founders Day 2019

Remembering its Founder-Director Dr Pushpa M Bhargava, the Centre for Cellular and Molecular Biology (CSIR-CCMB) celebrates his birthday February 22nd as its Founder's Day. The key features include popular talk by an eminent scientist or a distinguished personality and CCMB student's programme called Sci-Setu where CCMB

alumnus are invited to share their journey of success with the PhD students of CCMB. The event is closed with a cultural program followed by contributory dinner for the staff, students and their family members.

The 3rd Founders Day talk on *Health, Development and Equity: Can India bridge the gaps?* was delivered by Padma Bhushan Prof K Srinath Reddy, President, Public Health Foundation of India.

As part of Sci-setu, CCMB alumni, Dr T S Sridhar of 1986 batch, currently Professor and Head, Molecular Medicine at St. John's Research Institute, Bengaluru, was invited along with Dr Subhadeep Chatterjee of 1998 batch, currently Staff Scientist & Head, Laboratory of Plant-Microbe Interactions, Centre for DNA fingerprinting and Diagnostics, Hyderabad, to share their journey post-PhD.

The Founder's Day was closed with a cultural programme 'Tholu Bommalata' a traditional shadow puppet show of South India.

International Women's Day

In view of International Women's Day on 8th March 2019, CCMB Staff Club organized fun-based event for Women Employee of CCMB (Staff, Students & their family members).

2.2. I Science Outreach / Popularization Programs

STUDY TOURS

CCMB recieves a number of requests from Universities, Colleges and Schools every year for guided tours to the various facilities. CCMB took up this activity since its inception with an objective of keeping the young minds informed about the ongoing activities of CCMB in general and advances in frontier areas of modern biology in particular which they read in their text books in graduate and post-graduate studies. For this a guided tour will be organized to the visiting student groups. During the year 2018-19, 3438 students visited Centre from several colleges and Universities.

- Zilla Parishad School, Komorambheem Dist, Telengana
- Akshara The School, Hyderabad
- Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu
- Sagar Public School, Bhopal, Madhya Pradesh
- Hindusthan College of Arts and Science, Coimbatore, Tamil Nadu
- Biology Teachers from Mahabubnagar Dist, Telengana
- Prince Shri Venkateshwara Arts & Science College, Chennai, Tamil Nadu
- Biology Teachers from Wanaparthy Dist, Telengana
- Kalinga Institute of Industrial Technology, Bhubaneswar, Orissa
- P C Jabin Science College, Hubballi, Karnataka
- Tumkur University, Tumakurru, Karnataka
- Junior Civil Judges, Hyderabad
- College of Agricultural Bio Technology, Ratnagiri, Maharashtra
- Vels Institute of Science Technology, Chennai, Tamil Nadu
- Tumkur University, Tumakaru, Karnataka
- College of Agricultural Bio Technology, Ratnagiri, Maharashtra
- Sri Amogha Junior College, Hyderabad
- K T N College of Pharmacy, Thrissur, Kerala
- College of Veterinary & Animal Sciences, Parbhani, Maharashtra
- Indian Academy Degree College, Bangalore, Karnataka
- Nalanda College of Pharmacy, Nalgonda, Telengana
- NMKRV College for Women, Bangalore, Karnataka
- Genesis School, Hyderabad
- SDP-WLF at LaCONES, Hyderabad
- Gowtham Model School, Hyderabad
- Academic Heights Public School, Hyderabad
- St Thomas College, Trichur, Kerala
- Kumadvathi Science & Commerce PU College, Shimoga, Karnataka
- St Joseph College, Tiruchirapalli, Tamil Nadu
- Bangalore University, Bangalore, Karnataka
- Guru Jambheswar University, Hisar, Haryana
- Govt school from Jagithyal Dist, Telengana
- St Aloysius College, Mangalore, Karnataka
- Junior Civil Judges, Hyderabad
- Nehru Arts and Science College, Coimbatore, Tamil Nadu
- M R P G College, Vizianagaram, Andhra Pradesh
- NASR Boys school, Hyderabad
- Ch.S.D. St. Theresa's College for Women, Eluru, Andhra Pradesh
- Govt College of Arts, Sciences & Commerce, Goa
- Glendale Academy, Hyderabad
- Prof Jayashankar Telengana State Agricultural University, Hyderabad
- ICLE's Junjunwala College, Mumbai, Maharashtra
- Dr Yashwant Singh Parmar University of Horticulture, Hamirpur, Himachal Pradesh
- Savithribai Phule Pune University, Pune, Maharashtra
- B V Raju College, Bhimavaram, Andhra Pradesh
- Sangamner College, Sangemner, Maharashtra

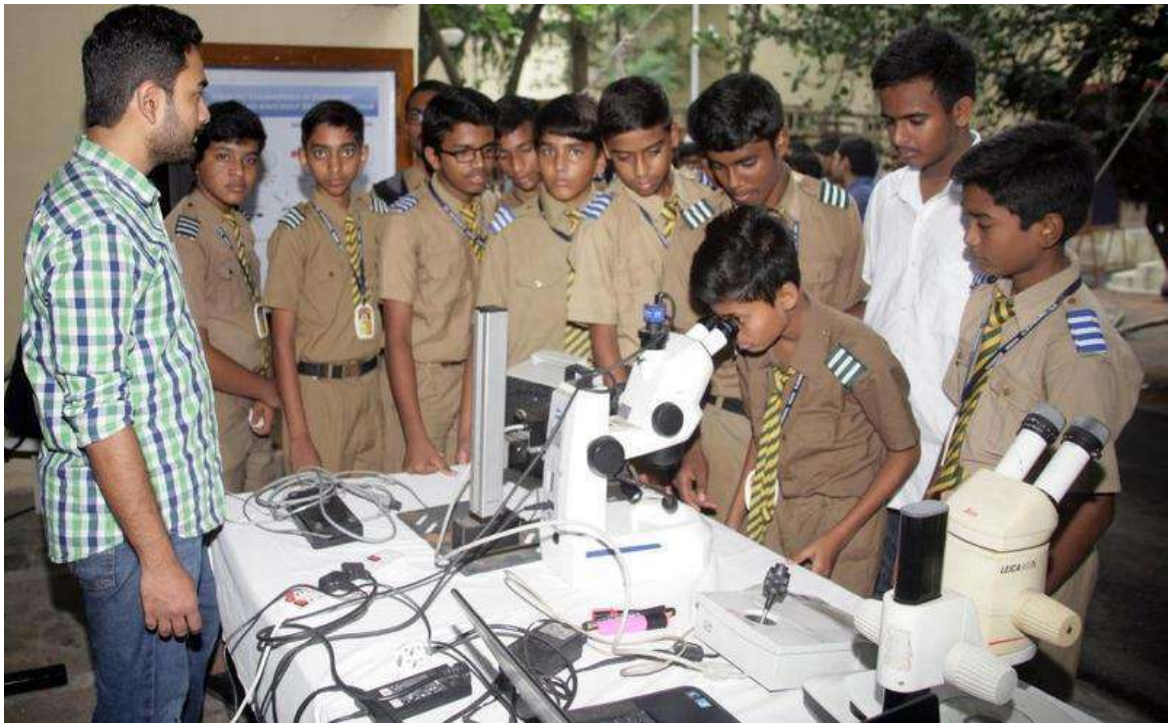
- Guru Nank Khalsa College, Mumbai, Maharashtra
- Telangana Social Welfare Residential Educational Institute, Jagitial, Telengana
- Madras Christian College, Chennai, Tamil Nadu
- Kashmir University, Srinagar, Jammu & Kashmir
- Dr Ambedkar College, Nagpur, Maharashtra
- Dr G R Damodaran College of Science, Coimbatore, Tamil Nadu
- Govt Degree College, Nirmal, Telengana
- Mahatma Gandhi Mission Institute of Health Science, Navi Mumbai, Maharashtra
- Mahatma Gandhi University, Kottayam, Kerala
- Orissa university of Agriculture & Technology, Bhubaneswar, Orissa
- Avanthi Group of Colleges, Hyderabad
- Shivaji University, Kolhapur, Maharashtra,
- College Teachers attending Refresher Course at University of Hyderabad
- Kerala University of Fisheries & Ocean Studies, Kochi, Kerala
- Modern College, Pune, Maharashtra
- Avanthi Institute of Pharmaceutical Sciences, Hyderabad
- Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, Maharashtra
- Bangurnagar Arts Science & Commerce College, Dandeli, Karnataka
- Madras Christian College, Tambaram, Chennai, Tamil Nadu
- Kathmandu University, Kavre, Nepal
- Sarojini Naidu Vanita Maha Vidyalaya, Hyderabad
- SV Agricultural College, Tirupati, Andhra Pradesh
- Davanagere University, Davanagere, Karnataka
- Eminent Biosciences, Indore, Madhya Pradesh
- I.D.S.G. Govt College, Chikkamagaluru, Karnataka
- Modern College, Pune, Maharashtra
- Govt College for Women, Thiruvananthapuram, Kerala
- Sree Narayana Guru College, Coimbatore, Tamil Nadu
- Kendriya Vidyalaya, Hyderabad
- Delhi Public School, Hyderabad
- Sri Bhavishyaa Educational Society, Hyderabad
- Abhaya School, Hyderabad
- Sri Ramakrishna College of Arts & Science for Women, Coimbatore, Tamil Nadu
- Mount Litera Zee School Manikonda, Telengana
- Zilla Parishad High School, NTR Nagar, Telengana
- Zilla Parishad High School, Budvel, Telengana
- Zilla Parishad High School, Vattinagulapally, Telengana
- Zilla Parishad High School, Kismatpur, Telengana
- Bharathidasan University, Tiruchirappalli, Tamil Nadu
- Sagar Public School, Bhopal, Madhya Pradesh

CCMB YOUNG INNOVATORS PROGRAM (YIP)

The Young Innovators Program (YIP) 2018-19 started with a public lecture by Dr Harsh Gupta, scientist at CSIR-National Geophysical Research Institute, Hyderabad. Of the 133 students who attended the lecture and appeared for the screening test, 24 were selected for a two week training program. The program provides the participants a flavour of what pursuing science is like, perform some of the classical experiments, and get trained in the scientific method of thinking. They visited different labs in CCMB, observed the work that happens there and interacted with the researchers who work in those labs in different campuses of CCMB. They did hands on experiments including isolating their own DNA from their cells, watching chromosomes, working with some of the common animal models. During these two weeks, the YIP participants were also provided an insight on different career opportunities in science, spanning from research to entrepreneurship.

CCMB OPEN DAY

CCMB celebrates CSIR's Foundation Day as its Open Day each year on 26th September, 2018. Around 12,000 visitors flocked to CCMB on this day in 2018. CCMB put up stalls and posters to explain the various research themes that it works on, and encouraged active discussions between the visitors and its researchers. In addition, the college students from the city who are trained in using Foldscope – the paper microscope, showed its use and utility to the visitors. There were 20 posters and 13 exhibits on various research programs of CSIR-CCMB, explained by our PhD students to the visitors.



SUPERHEROES AGAINST SUPERBUGS

CCMB has been the scientific partner for the Superheroes against Superbugs initiative. Started in Jul 2018, the initiative aims at creating champions for fighting antibiotic resistance among high school students. The team has developed lesson plans and activities to explain the emergence and impact of antibiotic resistance as a three day workshop. The team conducted their workshop with grade 9 students of two schools in Hyderabad with different socio-economic backgrounds – Global Edge, Madhapur and Telangana Social Welfare Residential Educational Institute School, Gowlidoddi. The workshop also facilitated meetings with scientists and start-ups working on understanding microbial physiology. As a final outcome of these workshops, it encouraged the participants to develop comics and skits that convey their learning and action points to combat the problem.

During the Antibiotic Resistance Awareness Week, discussions were done with parents of Global Edge network of schools in Hyderabad. A talk was arranged for the middle and high school students of Global Edge, Kokapet. A skit was developed and performed by PhD students of CCMB for students of Kendriya Vidyalaya at the KV Science Festival at Uppal.

PROJECT ABHILASHA

CCMB runs a mentoring program for the undergraduate science students in Hyderabad called Project Abhilasha. As a part of the pilot run in 2018-19, CCMB mentored students from three government colleges in the city – Nizam's College, Koti Women's College and Government Degree College, Khairatabad. Two teams, with five students and a teacher in each, were paired with a PhD student from CCMB as their mentor. They were mentored on appreciating scientific method of discovery through critiquing and analyzing a research publication along with imbibing communication skills. As a final outcome of the program after six months of mentoring, the participants presented the research paper in various forms – posters, talks and skits. The program ran from September 2018 to Feb 2019. During this program, the participants could also meet and interact with scientists and start-ups at CCMB and Atal Incubation Centre-CCMB, respectively.

ADHBHUTA

CCMB partnered with Ajahn Educational Foundation's Adhbhuta program. As a part of this initiative, CCMB invited around 800 middle and high school students from 10 schools across 5 days in November 2018 at its LaCONES campus. For the five days, CCMB ran sessions on the following:

- Cells as living machines,
- Why save Tigers
- Emergence of antibiotic resistance,
- Our roles in protecting sparrows by Dr Rajani Vakkalanka, Founder, Citizens' Action for Local Biodiversity Awareness and Conservation (CALBAC)

The sessions aimed towards inspiring the participants towards understanding the cellular and molecular details of how our bodies function as well as individual action points that can help us control and combat urging social causes such as wildlife conservation and fighting antibiotic resistance.

URBAN WILDLIFE

PhD students from LaCONES have designed sessions with middle school students on sensitizing them on urban wildlife. They conducted these sessions with two schools in Feb-Mar 2019. The sessions involve games to observe the surroundings for wildlife and their habitats during a nature walk, and to understand their behaviour better. The sessions also have detailed discussions with the ecology researchers who work at LaCONES on their work and how they hope to conserve ecosystems.

WILDLIFE WEEK CELEBRATIONS

CCMB celebrated the Wildlife Week on Oct 4-5th, 2018 by inviting school students for a wildlife quiz and drawing/painting competition at its LaCONES and CCMB main building respectively.

2.2. J Electronic and Social Media

Video series on Youtube

CCMB collected questions from school students based on their curiosity when they visited the campus during the Open Day. These questions were broadly classified into groups, and CCMB now runs a video series on its YouTube channel (CSIR-Centre for Cellular and Molecular Biology) called 'What the Science'. CCMB's PhD students explain the fundamentals of life sciences and much of the history of advancement in life sciences through this series of videos. The aim of this initiative is to popularize the logic with which life sciences is studied, and debunk that it is a subject of rote memorization.

Monthly newsletters

Since Jul 2018, CCMB runs a monthly newsletter – CCMB Daak that one can subscribe to via email. The newsletter aims to collate news of research and other activities and events in CCMB and Hyderabad, in general – that would interest a life science enthusiast.

Social media

CCMB actively engages with general public through its social media handles on Facebook, Twitter and LinkedIn. Through social media it reaches out to more than 10K followers.

School and college visits by scientists

PhD Students, Scientists and Technical Officers at CCMB visit local schools and colleges regularly to conduct sessions on the cutting edge science that the institute works on, different social issues where scientific knowledge and expertise can help, and inform them about different careers in sciences, particularly in life sciences.

2.2. K Staff, Research Students, Project Staff & Others

SCIENTIFIC RESEARCH GROUPS

Amit Asthana Group

Amit Asthana	Principal Scientist
Ira Bhatnagar	Senior Scientist
Naga Sowmya	Project Assistant-I

A S Sreedhar Group

A S Sreedhar	Principal Scientist
A Vijaya Lakshmi	Principal Scientist
K R Paithankar	Principal Technical Officer
Akhil Kotwal	Ph.D. student
Pankaj Kumar	Ph.D. student
Shrikant Dharaskar	Ph.D. student
Ramkumar Balaji	Project JRF

Purnima Bhargava Group

Purnima Bhargava	Emeritus Scientist

Venkata R Aditya Chalamcharla Group

Venkata R Aditya Chalamcharla	Project Scientist
Anubhav Bhardwaj	Technical Officer
Harsh Kapoor	PhD student
Annapoorna K P	PhD student

G R Chandak Group

G R Chandak	Senior Principal Scientist
K Radha Mani	Principal Tech Officer
Seema Bhaskar	Principal Tech Officer
Inder Deo Mali	Lab Assistant
P Ashok	Lab Assistant
Prachand Issarapu	Ph.D. student
Ashutosh Singh Tomar	Ph.D. student
Sara Sajjadi	Ph.D. student
Swathi Bayyana	Ph.D. student
Ajay Deepak Verma	Research Associate-I
Arumalla Manisha	Project Assistant-II
Mobeen Shaik	Project Assistant-II
Shoma Kumaresh Naskar	Project Assistant-II
Vinay Donipadi	Project Assistant-II
Punya Sri PSKDB	Project Assistant-I
Shagufta Tasneem	Project Assistant-I
Challapalli Mounika	Project JRF
Akshay Dedaniya	Project JRF

Amitabha Chattopadhyay Group

Amitabha Chattopadhyay	J C Bose Fellow
Parijat Sarkar	Ph.D. student
Bhagyashree D Rao	Ph.D. student
Sreetama Pal	Ph.D. student
Ashwani Sharma	Ph.D. student
Subhashree S Sahu	Project JRF
Aditya Kumar G	Project Fellow
Sarosh N Fatakia	Visiting Scientist

Ch Mohan Rao Group

Ch Mohan Rao	J C Bose Fellow
Kranthi Kiran Akula	Ph.D. student
Budnar Prashanth	Ph.D. student
Kamakshi Dandu	Ph.D. student

Mandar V Deshmukh Group

Mandar V Deshmukh	Principal Scientist
Upasana Rai	Ph.D. student
Sneha Paturi	Ph.D. student
Jaydeep Paul	Ph.D. student
Aute Ramdas A	Ph.D. student
Debadutta Patra	Ph.D. student

Jyotsna Dhawan Group

Jyotsna Dhawan	Chief Scientist
Sujoy Deb	Ph.D. student
Debarya Saha	Ph.D. student
Swetha S	Ph.D. student
Priti A S	Ph.D. student
Ananga Ghosh	Ph.D. student
Lamuk Zaveri	Project Fellow
GunjanPurohit	Research Associate-III
PrabhavathyDevan	Research Associate-I

G Umapathy Group

G Umapathy	Principal Scientist
Vinod Kumar	Technical Officer
Mihir Trivedi	Ph.D. student
Manu S	Ph.D. student
Krupa Vinay Teja P	Ph.D. student
G Anusha	Ph.D. student
Gopikrishnan P	Ph.D. student

Ajay Gaur Group

Ajay Gaur	Principal Scientist
A Sreenivas	Senior Tech Officer (1)

H H Krishnan Group

H H Krishnan	Senior Scientist
M Mohan Singh	Technical Assistant
Amit Kumar	Technical Assistant
Sana Parveen	Ph.D. student
Dhiviya V	Ph.D. student
Haripriya Parthasarathy	Ph.D. student
Divya Gupta	Ph.D. student
Vishal Sah	Ph.D. student
Poonam Manral	Project JRF

K Thangaraj Group

K Thangaraj	Chief Scientist

Nitin C Tupperwar	Senior Scientist (on lien)
G Mala	Principal Tech Officer
S Deepa Selvi Rani	Senior Tech Officer (2)
Jagamohan Chhatai	Technical Assistant
Ch Viswanatham	Lab Assistant
Sunil Kumar Tripathi	Ph.D. student
Rajan Kumar Jha	Ph.D. student
Nipa Basak	Ph.D. student
Jaydeep A Badarukhiya	Ph.D. student
Lomous Kumar	Ph.D. student
Umesh Kumar	Ph.D. student
Deepak Kumar K	Ph.D. student
Sagnik Dhar	Ph.D. student
Partheusa Machha	Ph.D. student
Alok Kumar	Ph.D. student
Rajesh V Iyer	Ph.D. student
Niranjani	Research Associate-I
Sivapriya Pavuluri	Research Associate-I
Chilukoti Neeraja	Research Associate-I
Narmada	Research Associate-I
Aishwarya Dhall	Project JRF

Arvind Kumar Group

Arvind Kumar	Principal Scientist
Sachin Singh	Scientist
Avijeet Kamle	DST-Project Investigator
Shams Ul-Haq Talee	Ph.D. student
Unis Ahmad Bhatt	Ph.D. student
Annapoorna P K	Ph.D. student
Niharika Awasthi	Ph.D. student
Aditya Undru	Ph.D. student
Bhanu Pranav N S	Ph.D. student
Gajendra Reddy	Research Associate-I

Lekha Dinesh Kumar Group

Lekha Dinesh Kumar	Principal Scientist

Megha Kumar Group

Megha Kumar	DST-INSPIRE Faculty
Sharda Ravi Iyer	Ph.D. student

Santosh Kumar Group

Santosh Kumar	Project Scientist
Sitanshu Kumar S	Ph.D. student
Ketaki Bhagwat	Ph.D. student
Rini Jacob	Research Associate-I

MukeshLodha Group

Mukesh Lodha	DBT Ramalingaswami Fellow
Akanksha Garhewal	Ph.D. student
Preethi Jampala	Ph.D. student
Shraddha Vijay Lahoti	Ph.D. student
Isha Joshi	Project JRF

M Mohammed Idris Group

M Mohammed Idris	Principal Scientist
Varsha Varakantham	Pharmacopoeia Scientist
Sareena Banu	Pharmacopoeia Associate
Chandani Tarachand D	Pharmacopoeia Associate

M V Jagannadham Group

M V Jagannadham	Chief Scientist
Deepika Chandra	Ph.D. student

Rakesh Kumar Mishra Group

Rakesh Kumar Mishra	Director
Rashmi U Pathak	Principal Scientist
A Srinivasan	Senior Tech Officer (1)
Runa Hamid	DST-Project Investigator
DivyaTej Sowpati	Scientist
Shagufta Khan	Ph.D. student
Phanindhar K	Ph.D. student
Nikhil Hajirnis	Ph.D. student
Ashish Bihani	Ph.D. student
Ravina Saini	Ph.D. student
Avvaru Akshay Kumar	Ph.D. student
Soujanya M S	Ph.D. student
Sonu Yadav	Ph.D. student
Shreekant Verma	Research Associate-I
Nisha M	Office Assistant

P Chandra Shekar Group

P Chandra Shekar	Senior Scientist
K Hanuman Tulashiram	Ph.D. student
Debabrata Jana	Ph.D. student
Vishnu Vijay	Ph.D. student
Mansi Srivastava	Ph.D. student
Rajendra Singh R	Project JRF

Anant B Patel Group

Anant B Patel	Principal Scientist
K S Vardarajan	Technical Officer
Narayan Datt Soni	Ph.D. student
Dipak Roy	Ph.D. student
Bedaballi Dey	Ph.D. student
Kamal Saba	Ph.D. student
Ajay Sarawagi	Ph.D. student
Akhila Ramesh	Project JRF

Hitendra Kumar Patel Group

Hitendra Kumar Patel	Senior Scientist
Raju Madanala	Senior Tech Officer (2)
B Kranthi	Technical Officer
Rajkanwar Nathawat	Ph.D. student
Vishnu Narayanan M	Ph.D. student
Kamal Kumar Malukani	PDF
Shakuntala E Pillai	Ph.D. student
Neha Rajendra K	Ph.D. student
Sohini Deb	Ph.D. student
Komal Ashok Awalellu	Ph.D. student
Gokulan C G	Ph.D. student
Roshan M V	Research Associate-I
Namami Gaur	Project Assistant-II
Shailaja Kanumuri	Project Assistant-I

R Nagaraj Group

R Nagaraj	J C Bose Fellow
Taniya Mary Binny	Project JRF

Swasti Raychaudhuri Group

Swasti Raychaudhuri	DBT Ramalingaswami Fellow
Shivali Rawat	Ph.D. student
Shemin Mansuri	Ph.D. student
Harshit Vaish	Ph.D. student
Pooja Ramesh Gupta	Ph.D. student
Sharmila Singh	Research Associate-I
Debodyuti Mondal	Project Assistant-II
Suparna Ghosh	Project JRF

Manjula Reddy Group

Manjula Reddy	Chief Scientist
G S N Reddy	Principal Tech Officer
S Venugopal	Senior Technician (2)
Nilanjan Som	Ph.D. student
Pavan Kumar Ch	Ph.D. student
Raj Bahadur	Ph.D. student
Shambhavi Garde	Ph.D. student
Moneca Kaul	Ph.D. student
Suraj Kumar Meher	Ph.D. student
Vaidehi Mihir Rajguru	Ph.D. student
RichaKhanna	PDF
Balaji V	Research Associate-II

Kumaraswamy Regalla Group

Kumaraswamy Regalla	Wellcome Trust DBT Fellow
Abishek Bharadwaj	Ph.D. student
Priyanka Pant	Ph.D. student
Disha Nanda	Ph.D. student
Manisha Kumari Sahu	Project JRF

Rajan Sankaranarayanan Group

R Sankaranarayanan	Chief Scientist
P Shobha Krupa Rani	Principal Scientist
Biswajit Pal	Senior Scientist
R Rukmini	Principal Tech Officer
P Sambhavi	Technical Officer
K Mallesham	Technical Assistant
Mazeed Mohammad	Ph.D. student
Patil Gajanan Shrikant	Ph.D. student
Santosh Kumar K	Ph.D. student
Jotin Gogoi	Ph.D. student
Sudipta Mondal	Ph.D. student
Pradeep Kumar	Ph.D. student
Sakshi Shambhavi	Ph.D. student
Koushick S	Ph.D. student
Raghvendra Singh	Research Associate-I
K Priyadarshan	Research Associate-I
Akshay Bhatnagar	Research Associate-I
Gurumoorthy Amudhan	Project Assistant-II
Priyanka Dahate	Project JRF
Vinitha Lakshmi V	Project JRF
Surabhi Pramanik	Project JRF

Yogendra Sharma Group

Yogendra Sharma	Chief Scientist
Syed Sayeed Abdul	Lab Attendant (2)
Asmita D Pawar	Ph.D. student
Radhika Khandelwal	Ph.D. student
Amrutha H C	Ph.D. student
Sai Uday Kiran P	Ph.D. student
VenuSankeshi	Research Associate-I

Imran Siddiqi Group

Imran Siddiqi	J C Bose Fellow
Aswan Nalli	Ph.D. student
A V Pardha Sardhi	Ph.D. student
Frank Keith Max	Ph.D. student
Survi Mahesh	Ph.D. student
Sivakumar P	Ph.D. student
G Saee Subhash	Ph.D. student
S Prashanthi	Office Assistant

Puran Singh Sijwali Group

Puran Singh Sijwali	Principal Scientist
S Thanumalayan	Senior Tech Officer (2)
Renu Sudhakar	Ph.D. student
Manish Bhattacharjee	Ph.D. student
Deepak Kumar	Ph.D. student
Zeba Rizvi	Ph.D. student
Srinivas Reddy G	Ph.D. student
Chhavi Dhawar	Ph.D. student
Somesh Machhindra G	Ph.D. student
Prajakta Pramod Biyani	Ph.D. student
Neeradi Dinesh	Research Associate-II
Gokula Priya G	Research Associate-I
Divya Das	Project JRF
Navin Adhikari	Project JRF

Shashi Singh Group

Shashi Singh	Chief Scientist
Vijayishwar Singh	Project JRF

Sadanand D Sontakke Group

Mridula A Srinivas	Project Assistant-II

Ramesh V Sonti Group

Ramesh V Sonti	Chief Scientist (on lien)

Ghanshyam Swarup Group

Ghanshyam Swarup	J C Bose Fellow
A Kishore Raghawan	Ph.D. student
Shivranjani C Moharir	Ph.D. student
Sayyad Zuber W Q	Ph.D. student
Swetha Medchalmi	Research Associate-I
Rajashri Ramaswamy	Project JRF

Raghunand R Tirumalai Group

Raghunand R Tirumalai	Senior Scientist
Ravi Prasad Mukku	Ph.D. student

Shrish Tiwari Group

ShrishTiwari	Principal Scientist
Prachi Singh	Scientist
P Ramesh	Principal Tech Officer
Deepti Rao	Ph.D. student
Ram Chandra P	Project JRF

Tushar Vaidya Group

Tushar Vaidya	Senior Principal Scientist
Loka Ram Prasad	Ph.D. student
Pradyumna Swanand P	Ph.D. student
Devi Prasad V	Ph.D. student
S Satyajeet Sunil	Ph.D. student

Karthikeyan Vasudevan Group

Karthikeyan Vasudevan	Senior Principal Scientist
Sadanand D Sontakke	Principal Scientist
B Sambasiva Rao	Senior Scientist
P Anuradha Reddy	Senior Scientist
S Harika	Technical Officer
K Rajya Lakshmi	Technical Officer
Afsar Sogra	Lab Assistant
Snehalatha Vadigi	DST Inspire Faculty
Ashish Jha	Ph.D. student
Siddharth Bhatia	Ph.D. student
Gayathri Sreedharan	Ph.D. student
Ravi Kumar Singh	Ph.D. student
Saketh Murthy	Ph.D. student
Alka Sahu	Ph.D. student

V Radha Group

V Radha	Chief Scientist
Divya S	Ph.D. student
Ramulu Ch	Research Associate-I

Sunil Kumar Verma Group

Sunil Kumar Verma	Principal Scientist

Palani Murugan Rangasamy Group

P Murugan Rangasamy	Senior Scientist
Sanjay Kumar Suman	Technical Assistant
Ashis Kumar Pradhan	Ph.D. student
Ganapathi Kandasamy	Research Associate-I
Revathi M	Project JRF

Yelam Sreenivasulu Group

Yelam Sreenivasulu	Principal Scientist
V Vijaya Bhaskar	Principal Scientist
G Bhargavi Krishnasree	Ph.D. student

Sonal N Jaiswal Group

Sonal N Jaiswal	Ramanujan Fellow
J Nandan	Ph.D. student
K Aishwarya Arun	Ph.D. student
Titus S Ponrathnam	PDF
Priyanka Pandey	Project JRF

Pavithra Chavali Group

Pavithra Chavali	
Sourav Ganguli	Ph.D. student

Meghna Krishna Das Group

Meghna Krishna Das	Project Scientist

Jahnavi Joshi Group

Jahnavi Joshi	Project Scientist

S&T Resource Group

K Lakshmi Rao	Senior Principal Scientist
P Kavin Kennedy	Senior Principal Scientist
Sandeep Goel	Principal Scientist
Manoj Balyan	Senior Scientist
Suman S Thakur	Senior Scientist
A Sharada Devi	Principal Tech Officer
Bh Muralikrishna	Principal Tech Officer
Sandeep Shrivastava	Senior Tech Officer (1)
R Phanindranath	Senior Tech Officer (1)
M Sanjeev C Nayak	Technical Officer
G Vidyasagar	Lab Attendant (2)

Innovation Cell

Tushar Vaidya	Scientist-in-charge
Archana B Siva	Principal Scientist
N Nagesh	Senior Principal Scientist
Jomini Liza Stephen	Principal Scientist
C B Tripura Sundari	Scientist
B Kiran Kumar	Scientist
V Srinivas	Principal Tech Officer
Y V Subba Lakshmi	Senior Tech Officer (2)
V Anuradha	Senior Technician (2)
Hemalatha	Senior Steno
K Srinath	Lab Attendant (2)
Challa Venkatapathi	Project Assistant-II
Gaddam Kiranmai	Project JRF

Diagnostics Facility

M B Madhavi	Technical Officer (I/c Diagnostics)
M K Kanakavalli	Senior Tech Officer (1)
O V Padmalatha	Senior Tech Officer (1)
Raghavendra Babu	Technician (1)
Karthik Bharadwaj	Principal Clinical Geneticist
V Jyothi	Project Assistant
M Pallavi	Project Assistant

CCMB-Atal Incubation Centre

N Madhusudhana Rao	Chief Executive Officer
Ramjee Pallela	Chief Operating Officer
Ritika Marrampalli	Communications Manager
YSSV Prasad	Job Contract

TECHNICAL GROUPS

RESEARCH FACILITIES

Animal House

M Jerald Mahesh Kumar	Principal Scientist
Jayashree Chiring Phukon	Scientist
A Rajasekharan	Principal Tech Officer
Jedy Jose	Senior Tech Officer (1)
N Sairam	Technical Assistant
T Sreeramulu	Senior Technician (2)
V Allaiah	Senior Technician (2)
S Prashanth	Technician (1)
B Yadagiri	Lab Assistant
P Ravi	Lab Assistant
R Ellesh	Lab Assistant
M Nageswara Rao	Lab Assistant
R Siddaramappa	Lab Assistant
K Raju	Lab Attendant (2)
B Lalaiah	Lab Attendant (2)
M Rajeshwari	Multitask Staff

Bioinformatics

Divya Tej Sowpati	Scientist
Nitesh Kumar Singh	Sr Tech Officer (1)
Sofia Banu	Project Assistant-II
Archana Verma	Project Assistant-II
Rama Sarvani Krovi	Project Assistant-II
Satuluri Sri Harsha	Project Assistant-II
Deepak Sharma	Project Assistant-II
Priya Singh	Project Assistant-II
Onkar Vasanthrao Kulkarni	Project Assistant-II
Abhijeet Karan	Project Assistant-II
Tanya Aggarwal	Project Assistant-II

Drosophila Facility

V Bharathi	Sr Tech Officer (2)
K Ramachandra Rao	Technical Officer
P Sabitha	Lab Attendant (2)

Imaging Facility

Nandini Rangaraj	Chief Scientist
C Subbalakshmi	Principal Tech Officer
G Srinivas	Sr Tech Officer (1)
Harikrishna Adicherla	Sr Tech Officer (1)
T Avinash Raj	Sr Tech Officer (1)
Suman Bhandari	Technical Assistant

Next Generation Sequencing Facility

Mohammad Jafurulla	Sr Tech Officer (1)
V Purushotham	Technical Assistant

Proteomics Facility

V Krishna Kumari	Principal Tech Officer
C Sivakama Sundari	Principal Tech Officer
B Raman	Principal Tech Officer
Y Kameshwari	Principal Tech Officer
K Ranjith Kumar	Technical Assistant

Tissue Culture Facility

Ch Varalakshmi	Principal Tech Officer
V R Sundereswaran	Principal Tech Officer
Zareena Begum	Principal Tech Officer
B V V Pardhasaradhi	Senior Tech Officer (3)
D Partha Sarathi	Senior Tech Officer (1)
S Easra	Senior Technician (2)
T Dayakar	Lab Attendant (1)

Transgenic Knockout Facility

B Jyothi Lakshmi	Senior Tech Officer (1)
S Purnima Sailasree	Technical Officer
AshaKumari	Technical Officer

Zebrafish Facility

M L Arvinda Swamy	Technical Officer

ACADEMICS

Academic Cell

Manjula Reddy	Coordinator
Puran Singh Sijwali	Coordinator
V Anitha	Office Assistant

SUPPORT FACILITIES

Planning, Monitoring and Evaluation (PME)

M R Vishnu Priya	Senior Principal Scientist
B V Ramakrishna	Senior Tech Officer (3)
K Satyanarayana	Lab Assistant
Gulzar Khan	Lab Attendant (2)

Business Development, Human Resources & Documentation Group

Archana B Siva	Principal Scientist
R Leela Kumari	Principal Tech Officer
Divya Singh	Business Development Officer
K Anitha	Technician (1)
Pallavi M	Project Assistant-II

Fine Biochemicals

Y Rama Dasu	Principal Tech Officer
Kishore Joshi	Senior Tech Officer (3)
M C Joseph	Lab Assistant

Instrumentation Group

I Asha Ramesh	Principal Tech Officer
Lora B Narayana	Principal Tech Officer
J D Fernandes	Principal Tech Officer
Mahesh Prasad	Senior Tech Officer (3)
U S T R B Bapi Raju	Senior Tech Officer (3)
B Venkata Narayana	Senior Tech Officer (3)
N R Chakravarthi	Senior Tech Officer (2)
Sudatt T Tambe	Senior Tech Officer (1)

Dattatrya N Gurkhel	Sr Tech Officer (1)
K Sanjeev Kumar	Sr Tech Officer (1)
A Bala Murugan	Sr Tech Officer (1)
A Syam Kumar	Sr Tech Officer (1)
Devender Sundi	Technical Officer
Chetan R Khapekar	Technical Assistant
Amol Mandlik	Technical Assistant
Angothu Ramesh	Technical Assistant

Information Technology (IT) Group

Geetha Thanu	Senior Scientist
Sublari Balaraju	Scientist
Aparna Kumari	Scientist
Biswajit Roy	Scientist
P Nagalinga Chary	Sr Tech Officer (3)
P Radhakrishna Murthy	Sr Tech Officer (3)
K Sambasiva Rao	Sr Tech Officer (2)
N Siva Rama Prasad	Sr Tech Officer (2)
A Padmavathi Devi	Sr Tech Officer (2)
S Mahalingam	Sr Tech Officer (2)
K Rama Chary	Sr Tech Officer (1)
Sreekanth Mamidala	Sr Tech Officer (1)
Y Padmavathi	Private Secretary
R Koteswara Rao	Senior Technician (2)
K Gopichand	Senior Technician (2)
M Srinivasa Rao	Lab Attendant (2)

Laboratory Technical Services & Horticulture

Y V Rama Rao	Senior Suptd Engineer
Mani Ramana Rao	Senior Steno
S Krishna	Lab Assistant
P Gyaneshwar	Lab Assistant
L Laxman Dora	Lab Assistant
M A Jaleel	Lab Assistant
B Sanjeeva Rao	Lab Assistant
M M Rajendran	Lab Assistant

Engineering Services

G C Thanu	Senior Suptd Engineer
Ch Bikshamaiah	Senior Suptd Engineer
Ashok Baswa	Senior Suptd Engineer
G Rajendra Prasad	Senior Suptd Engineer
Sheelwantayya	Suptd Engineer
Devidas M Nikhar	Asst Exe Engineer
B Vijaya Kumar	Asst Exe Engineer
K Nagendrababu	Asst Exe Engineer
A Varaprasada Rao	Asst Exe Engineer
V Prabhakar	Senior Technician (2)
S Sreehari	Senior Technician (2)
G Ramakrishna Chary	Senior Technician (2)
M P Premkumar	Senior Technician (2)
Ch Ravindra Babu	Senior Technician (2)
K Mohan	Senior Technician (2)
A Prem Kumar	Senior Technician (2)
M Tirumala Rao	Senior Technician (2)
Ananda S Pahurkar	Senior Technician (2)
Suresh Prasad	Senior Technician (2)
K Venkatramana	Senior Technician (2)
A J Narsing Rao	Senior Technician (1)
K Shankar	Senior Technician (1)
D Vinod Kumar	Technician (1)
L Kumar	Technician (1)
Anirban Adhikari	Technician (1)
Mallikanti Srinu	Technician (1)
G Ramesh	Assistant (G) Gr.I
S Venkata Sastry	Lab Assistant
P Venkatarama Rao	Lab Assistant
AV Ramakrishna Reddy	Lab Assistant
T VenkateswarRao	Lab Assistant
M Mazhar Ali	Lab Assistant
K Sreeram	Lab Assistant
M Padmanabhan	Lab Assistant
K Nagabhushanam	Lab Assistant
B M Nagesh	Lab Assistant
M Anjaneyulu	Lab Assistant
K Narayana	Lab Assistant
B Balakrishna Reddy	Lab Assistant
N NarasingaRao	Lab Assistant
Syed Khundmier	Lab Assistant
C Rosaiah	Lab Assistant
V Shankar Rao	Lab Assistant
B Satyanarayana	Lab Assistant
T Sambasiva Rao	Lab Attendant (2)
P Srinivas	Lab Attendant (1)

ADMINISTRATION & MANAGEMENT

Director's Office

D Lavanya	Senior Tech Officer (2)
B V N Naveen Kumar	Technical Officer
S Madhuri	Staff Officer & I/c Academic Cell
Somdatta Karak	Communications Officer
Surabhi Srivastava	Research Manager

Administration

P Sudha Rani	Administrative Officer
Y Srinivasa Rao	Administrative Officer
Noopur Rani Prasad	Hindi Officer
Sunil Kumar	SO (G)
J Venu	Senior Tech Officer (2)
M Ranga Rao	Senior Steno
S Mukhtar Ahmed	Assistant (G) Gr.I
S Kanchanamala	Assistant (G) Gr.I
R Gopal	Assistant (G) Gr.I
Vivek Khare	Assistant (G) Gr.I
Manju Singh	Assistant (G) Gr.I
T Rajani	Assistant (G) Gr.I
Ch Sridevi	Assistant (G) Gr.I
Ashok Kumar Swasani	Assistant (G) Gr.I
K Pratap	Assistant (G) Gr.II
Abdul Raheem Qureshi	Assistant (G) Gr.III
Savita Kumari	Junior Hindi Translator
K Madhavi	Receptionist
Mahendra	Senior Technician (2)
B Srinivas	Senior Technician (2)

K Satyanarayana	Senior Technician (2)
G Anand	Senior Technician (2)
Mohd Pasha	Senior Technician (2)
M Devendra Nath	Senior Technician (2)
D Ramesh	Senior Technician (2)
B Sadanandam	Senior Technician (2)
Mahender Vynala	Technician (1)
Mohd Gazanfar Ali	Lab Assistant
K Krishnamacharyulu	Lab Assistant
Ravindranath	Lab Assistant
B Venkateswarulu	Lab Assistant
Ch Chandrashekar	Lab Assistant
M Sharadha	Bearer (Adm)
C V S Padmaja	Bearer
Ambe Naveen Kumar	Multitask Staff

Finance & Accounts

S K Roy	FAO
Ch Vijaya	SO (F&A)
V V L Prasanna	Assistant (G) Gr.I
K Rama Krishna	Assistant (F&A) Gr.I
Vimala Prakash	Assistant (F&A) Gr.II
K Sujatha	Assistant (F&A) Gr.II
M V Subba Rao	Senior Steno
G Anuradha	Jr Secretarial Asst
W Sudhakar	Senior Technician (2)
M Vishnu Yadav	Technician (1)
K Venkateswarulu	Lab Assistant
Nagamani A K	Project Assistant-I

Stores & Purchase

Dharmendra Kumar	Stores &Purchase Officer
B Rajender Kumar	SO (S&P)
Govind Kumar Jha	Assistant (S&P) Gr.I
D V Ramana Murthy	Assistant (S&P) Gr.I
S Aruna	Assistant (S&P) Gr.I
S S Lakshmi	Assistant (S&P) Gr.III
N S Sandeep Kumar	Assistant (S&P) Gr.III
D Balaji Prasad	Senior Technician (2)
K Manik Rao	Senior Technician (2)
S Riyasat Ali	Senior Technician (1)
Maqsood Ali	Junior Steno
Sharif Abdul Aleem	Lab Assistant
Mohd Yakub Akheel	Lab Attendant (1)

Medical Services

G Sujatha	Medical Officer
V Venugopal Rao	Medical Officer
T Nagalakshmi	Technical Officer
A Mahesh	Technical Assistant
U V Sitaramamma	Senior Technician (2)
M R Ravindra Nath	Senior Technician (2)
R Palnitkar	Consultant
Ravinder Reddy D	Project Fellow

Security

C V Tirumala Rao	Senior Security Officer

Guest House

Anil Kumar Sahu	Principal Tech Officer
G Christy Wilson	Senior Technician (2)
A Selvam	Senior Technician (2)
Benedict	Senior Technician (2)
Mohd Jaffer	Lab Assistant

Canteen Group

Vikram Kumar	Technical Officer
M Venkatesan	Senior Technician (2)
P M Mani Maran	Senior Technician (2)
K Ramesh Babu	Senior Technician (2)
B M Narasing Rao	Lab Assistant
Mohd Athar Ali	Lab Assistant
N Aruna	Lab Assistant
R Suresh Kumar	Lab Assistant
S Yadaiah	Lab Attendant (2)
Savitri Luhura	Bearer

2.3 JONAKI-BRIT/DAE ^{32}P LABELLED BIOMOLECULES LABORATORY

The Labelled Biomolecules Laboratory, Regional Centre (RC), Jonaki, Board of Radiation & Isotope Technology (BRIT), Department of Atomic Energy, situated in the Centre for Cellular & Molecular Biology (CCMB) campus is serving the various national laboratories, universities, industrial research centres, and hospitals involved in biotechnology, agriculture, life sciences & medical research by providing ^{32}P labelled nucleotides since 1988.

We supply ^{35}S labelled amino acids and a range of ^{99m} Tc-radiopharmaceutical cold kits produced at Radiopharmaceutical laboratory of BRIT in Mumbai. Cold kits are for use in conjunction with ^{99m} Tc-Pertechnatate, in imaging of human organs for diagnosis and treatment, to the nuclear medicine centres of the hospitals and diagnostic centres in and around Andhra Pradesh. In order to expand the service we will soon begin supply of ^{99m} Tc sodium pertechnetate from radio-pharmacy laboratory at RC, JONAKI.

RC, JONAKI, BRIT has a patented FRET based qPCR chemistry which has been validated. Real time M.tb detection kit based on the above FRET technology have been developed and clinically evaluated in collaboration with Nizam's Institute of Medical Sciences (NIMS), Hyderabad. Proto type kits are under evaluation before they are introduced as regular products.

We supply Taq DNA polymerase, PCR master mix, and DNA Isolation kits across the country on a regular basis.

LIST OF PRODUCTS
RADIOACTIVE BIOCHEMICALS

1. ^{32}P Nucleotides:

CODE	PRODUCT
101	[γ ^{32}P] ATP
102	[α ^{32}P] dCTP
103	[α ^{32}P] dATP
104	[α ^{32}P] dGTP
106	[α ^{32}P] ATP
107	[α ^{32}P] GTP
108	[α ^{32}P] UTP
109	[α ^{32}P] CTP
1010	[3'5'-α ^{32}P] pCP
1011	[γ ^{32}P] GTP
LCP 32	[^{32}P]-Orthophosphoric Acid

The above products are available in two formulations (dry ice and ambient temperature shipments) fortnightly.

2. ^{35}S Amino Acids

CODE	PRODUCT
LCS 1/LCS 2	^{35}S Methionine
LCS 3	^{35}S Cysteine
LCS 7	^{35}S Methionine-Cysteine mix Eleg mix
LCS 6	^{35}S Glutathione
LCS 8	Protein in vivo twin label mix

NON-RADIOACTIVE BIOCHEMICALS

CODE	PRODUCT
LCK-1	Nick Translation Kit
LCK-2	Random Primer Kit
LCK-1601	dNTP mix for PCR (1 set of 4 dNTPs in 4 x 25 µl)
LCK-1602	dNTP mix for PCR (3 set of 4 dNTPs in 4 x 25 µl)
LCK-1603	dNTP mix for PCR (5 set of 4 dNTPs in 4 x 25 µl)
LCK-1604	dNTP mix for PCR (10 set of 4 dNTPs in 4 x 25 µl)
LCE-101	Taq DNA Polymerase Enzyme (100 Units)
LCE-102	Taq DNA Polymerase Enzyme (250 & 500 Units)
LCE-103	Taq DNA Polymerase Enzyme (1000-4000 Units)
LCE 104	Taq DNA Polymerase Enzyme (5000-50000 Units)
LCE 105	Taq DNA Polymerase Enzyme (60000 up to 90000 Units)
LCE 1000	Bulk packs more than 100000 units on enquiry
PMX 01	PCR Master Mix [100 Rxn (2 x 50)]
PMX 02	PCR Master Mix [250 Rxn (5 x 50)]
PMX 05	PCR Master Mix [500 Rxn (5 x 100)]
PMX 10	PCR Master Mix [1000 Rxn (5 x 200)]
PMX 1000	PCR Master Mix (On enquiry)
LCK1701	*M.tuberculosis* PCR detection kit (25 reaction kit)
LCK 1702	*M.tuberculosis* PCR detection kit (50 reaction kit)
LCK 20	Genomic DNA Isolation kit (50 reaction kit)
LCK 21	Genomic DNA Isolation kit (100 reaction kit)

LCK 22	DNA Isolation kit (Plasmid) (50 reaction kit)
LCK 23	DNA Isolation kit (Plasmid) (100 reaction kit)
LCK 24	DNA Gel Purification kit (50 reaction kit)
LCK 25	DNA Gel Purification kit (100 reaction kit)
LCK 26	PCR Product Purification kit (50 reaction kit)
LCK 27	PCR Product Purification kit (100 reaction kit)

Cold kits for formulation of ^{99m}Tc-radiopharmaceuticals

Code	Kit for preparation of TC-labelled Short form Radiopharmaceutical
TCK-5	Sulphur Colloid ^{99m}Tc S/C
TCK-7	Diethylene Triamino Penta Acetic Acid ^{99m}Tc-DTPA
TCK-15	Glucoheptonate ^{99m}Tc-GHA
TCK-16	Phytate ^{99m}Tc-Phy
TCK-30	Methylene DiPhosphonate ^{99m}Tc-MDP
TCK-33	DMSA Injection ^{99m}Tc-(III) DMSA
TCK-35	DMSA ^{99m}Tc-DMSA
TCK-38	Stannous – pyrophosphate Sn-pyp
TCK-39	Mebrofenin ^{99m} Tc-Mebro
TCK-42	Ethyl cystinate dimer ^{99m}Tc ECD
TCK-43	Ethylene di cysteine ^{99m}Tc-EC
TCK-50	MIBI ^{99m}Tc-Mibi
TCK-52	Tetrofosmin (lyophilised) ^{99m}Tc-Tetrofosmin
TCK-53	HSA- nanocolloid ^{99m}Tc- HSA-NC
LUK-1	177Lu-EDTMP 177Lu-EDTMP

Staff of JONAKI (as on 31-03-2019)

1. SMT PAPIA HAZRA, OIC, RC HYDERABAD
2. DR. B.R. VARMA, MANAGER
3. DR. T.K.SANKARANARAYANAN, MANAGER
4. SHRI N. AMBEDKAR
5. SHRI M. SRINEEVASULU
6. SHRI S. SRIKANTH
7. SHRI T.K. SUDHIR
8. SMT. T. RAJA RAJESWARI
9. SHRI M.B. KUMBHAR
10. SHRI P.B. MOREY
11. SHRI JAGDISH CHANDRA
12. SHRI S. VENKATESH
13. SHRI YAKUB ALI

Order for all products can be directly placed with:

OFFICER-IN-CHARGE,
REGIONAL CENTRE, JONAKI, BRIT,
CCMB CAMPUS, UPPAL ROAD,
HYDERABAD-500 007.

E mail: rcrhyderabad@britatom.gov.in