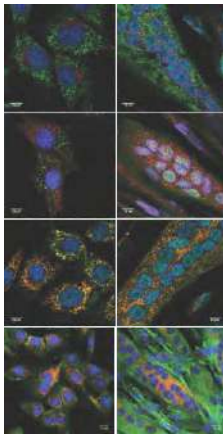
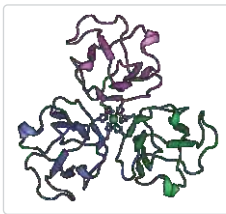


**Annual Report
2016 - 2017**



Front Cover

Expression of quiescence markers identified from cultured myoblasts in quiescent muscle stem cells. [For details see page (35)]



Back Cover

Crystal structure of the $\beta\gamma$ - Crystallin "Methallin" from the Methanoarchaea *Methanosaeta thermophila* (For details see page 105).

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Annual Report

2016 - 2017



**CSIR-Centre for Cellular and Molecular Biology
Hyderabad**

Contents

प्रस्तावना

Foreword

I. Charter	3
II. Research Programmes	5
Ramesh Kumar Aggarwal (Molecular Genetics and Developmental biology)	7
Amit Asthana (Applications of microfluidics, micro and nanotechnology in life sciences)	11
A S Sreedhar (Stress biology and molecular medicine)	14
Purnima Bhargava (Epigenetic mechanisms of gene regulation)	17
Venkataditya R Chalamcharla (Regulation of gene expression - by affecting the production or stability of RNA)	20
Giriraj Ratan Chandak (Complex biology and human reproductive genetics)	21
Amitabha Chattopadhyay (Membrane and receptor biology)	26
Mandar V Deshmukh (Post-transcriptional Gene Silencing Mediated by RNA-Binding Proteins)	30
Jyotsna Dhawan (Molecular Programs of Quiescence in Adult Stem Cells and Regeneration)	34
G Umapathy (Studies on Species Extinction and Conservation Physiology)	38
Ajay Gaur (Conservation Genetics of Endangered Species of India)	42
Krishnan H Harshan (Regulation of Protein Translation In Viral infections and biological systems)	45

K Guruprasad (Protein Sequence, Structure Analysis and Drug Design)	48
K Thangaraj (Evolutionary and Medical Genetics)	50
Lakshmi Rao Kandukuri (Chromosome Biology and Human Reproductive Genetics)	54
Arvind Kumar (Non-coding RNAs in diverse brain regions in stress response and depression)	57
Lekha Dinesh Kumar (Role of <i>wnt</i> signalling in EMT and development of colon cancer)	59
Satish Kumar (Functional Genomics using Transgenic and Knockout Mice and Molecular Approaches in Animal Breeding)	62
Mukesh Lodha (Mechanism of Epigenetic Inheritance in Plants)	65
M M Idris (Bio-mechanisms of Regeneration and Degeneration)	67
M V Jagannadham (Studies on outer membrane vesicles of bacteria)	69
Rakesh K Mishra (Genome Organization and Epigenetic Regulation)	72
P Chandra Shekar (Early embryonic development in mouse)	77
Veena K Parnaik (Nuclear organization and lamin biology)	79
Anant B Patel (¹³ C Nuclear Magnetic Resonance Investigations of Neurotransmitter Energetics in Neurological Disorders)	81
R Nagaraj Host-defense Antimicrobial Peptides; Activity and Developing Future Therapeutic Agents	85

Palani Murugan Rangasamy (The Regulation of Polyamine Homeostasis and their relevance in Health and Diseases of Eukaryotes)	88
Ch Mohan Rao (Molecular chaperones in health and diseases & Molecular diagnostics, therapeutics and drug delivery)	90
Swasti Raychaudhuri (Proteotoxicity in age-related diseases)	95
Manjula Reddy (Bacterial cell wall synthesis and its regulation)	98
Kumaraswamy Regalla (Non-coding RNAs in patho-physiology of the heart)	100
Rajan Sankaranarayanan (Structural Biology)	102
Yogendra Sharma (Calcium signaling via calcium-binding proteins)	106
Imran Siddiqi (Plant Reproductive Biology)	108
Puran Singh Sijwali (Roles of the ubiquitin proteasome system and autophagy in malaria parasite biology and pathogenesis)	110
Shashi Singh (Adult Stem Cells from Various Sources and Tissue Engineering)	113
Sadanand D Sontakke Reproductive Biotechnologies for Wildlife Conservation)	115
Ramesh V Sonti (Plant-Pathogen Interactions)	117
Ghanshyam Swarup (Molecular mechanisms of neuro-degeneration caused by mutations in optineurin)	120
Raghunand R Tirumalai (Dissecting the Molecular Basis of Mycobacterium tuberculosis Pathogenesis)	123

Shrish Tiwari (Sequence analysis of biomolecules)	126
Tushar Vaidya (Molecular analysis of host-pathogen interactions)	128
Karthikeyan Vasudevan (Ecology and conservation of endangered species)	132
Vegesna Radha (Signaling to the cytoskeleton and regulation of cellular functions)	136
Sunil Kumar Verma (Signal Transduction Studies in Human health and Disease and Molecular Biology Applications in Wildlife Conservation)	139
III.1 List of Publications and Patents	141
III.2 PhD Program	153
III.3 Training Programs	154
III.4 Research Facilities	155
III.5 Research Resources	161
III.6 Services	164
III.7 CCMB Innovation Hub	166
IV. Administration and Management	167
V. General Information	171
VI. JONAKI-BRIT/DAE 32P LABELLED BIOMOLECULES LABORATORY	199



प्रस्तावना



प्रस्तावना

वर्ष 2016-2017 के लिए वार्षिक रिपोर्ट प्रस्तुत करते हुए मुझे अत्यंत प्रसन्नता हो रही है। इस रिपोर्ट में पिछले वर्ष सीएसआईआर - सीसीएमबी की अनुसंधान, शिक्षा और प्रशिक्षण गतिविधियों पर प्रकाश डाला गया है।

सीसीएमबी ने जीव विज्ञान के विभिन्न क्षेत्रों में अनुसंधान करता रहा है जिसमें (i) कोशिका एवं भ्रूण विकास, (ii) आनुवंशिक, जीनोमिक्स एवं एपिजेनेटिक्स, (iii) संक्रामक रोग, (iv) पादप जैविकी एवं कृषि तकनीक, (v) संरचनात्मक जैविकी, (vi) वन्यजीव संरक्षण और पारिस्थितिकी, आदि प्रमुख रहे हैं।

इन शोध गतिविधियों के अलावा, सीसीएमबी उन क्षेत्रों में सक्रिय रूप से काम कर रहा है

जिनका सामाजिक पर तत्काल प्रभाव पड़ता है। इसमें चावल की उन्नत किस्म (सीएसआईआर-सीसीएमबी और आईसीएआर-आईआईआरआर द्वारा संयुक्त रूप से विकसित) का प्रचार-प्रसार शामिल है जो अबतक देश के सात राज्यों में अपनाया जा चुका है। एक अन्य गतिविधि के अंतर्गत वन्यजीव फोरेंसिक और डीएनए डायग्नोस्टिक सेवाओं को उपलब्ध किया जा रहा है। इस ओर हमारी तीसरी गतिविधि तेलंगाना और आंध्र प्रदेश के चयनित क्षेत्रों में सेब की खेती का आरंभ एवं प्रसार है।

भारत में अपने युवाशक्ति का सबसे बड़ा अनुपात है। इस बात का फायदा जैव प्रौद्योगिकी उद्योग के लिए कुशल मानव संसाधन बनाने के लिए उठाया जा सकता है। मेरा मानना है कि ये ऐसे क्षेत्र हैं जहां

सीसीएमबी महत्वपूर्ण योगदान देने के लिए अच्छी तरह से सक्षम है। सीसीएमबी ने उद्योग की जरूरतों के लिए तथा चिकित्सा-छात्रों को अनुसंधान क्षेत्र में प्रोत्साहित करने तथा कौशल विकास के लिए कई प्रशिक्षण कार्यक्रम शुरू किए हैं।

आज का विज्ञान कल की सामाजिक-आर्थिक गतिविधियों के लिए एजेंडा निर्धारित करता है। इसलिए, विज्ञान के संदेश, अभूतपूर्व संभावनाओं एवं समाज के विविध मुद्दों पर जागरूपकता फैलाना अत्यंत महत्वपूर्ण है। सीसीएमबी इस उद्देश्य को पूरा करने के लिए प्रतिबद्ध है। इस ओर हम हर वर्ष सीसीएमबी में ऐसे अनेक कार्यक्रम आयोजित करते हैं जिसके माध्यम से देश भर के युवा छात्रों एवं सामान्य जनता के साथ परस्पर संबंध स्थापित कर सकें। इनमें प्रयोगशाला का

सार्वजनिक दिवस आयोजन, शैक्षणिक भ्रमण, पुलिस, वन, विधि तथा उद्योग से संबंधित लोगों द्वारा प्रयोगशाला भ्रमण शामिल हैं।

प्रौद्योगिकियों का स्वरूप बदल रहा है तथा नए रूप से उभरती हुई संभावनाएं और चुनौतियां सीएसआईआर-सीसीएमबी जैसी प्रयोगशालाओं को आगे की पंक्ति में रखती हैं जहां हम से राष्ट्र की जरूरतों और आकांक्षाओं को पूरा करने की उम्मीद होती है। मुझे इसमें कोई संदेह नहीं है कि सीसीएमबी इस चुनौतियों का सामना करने के लिए पूरी तरह

राकेश कुमार मिश्र
निदेशक

The background features a faint, light-colored ribbon diagram of a protein structure. Two decorative swooshes, one yellow and one blue, curve around the central text.

Foreword



Foreword

It is a great pleasure to present the Annual Report for the year 2016-2017. The report highlights the research, education and training activities of CSIR-CCMB during the past year.

CCMB carries out research in diverse areas of biology that include (i) Cell and Developmental Biology, (ii) Genetics, Genomics and Epigenetics, (iii) Infectious Diseases, (iv) Plant Biology and Crop Technologies, (v) Structural Biology, (vi) Wildlife Conservation and Ecology.

In addition to these research activities, CCMB is actively involved in the areas that have immediate societal impact. These include popularization of improved rice variety (developed jointly by CSIR-CCMB and ICAR-IIRR) that has now reached into seven

states of the country. Another activity is offering wildlife forensic and DNA diagnostic services. The third such activity in this area is popularization of apple cultivation in selected regions of Telangana and Andhra Pradesh. There are plans to upscale these activities for more societal benefits.

India has to take full advantage of its unique position of being a nation with the largest proportion of young people. Skilled human resource for the biotechnology industry and addressing our health issues at a large scale are going to be important. I believe that these are the areas where CCMB is very well poised to make significant contributions. CCMB has initiated several training programmes for skill development for industry needs and to encourage research interest in medical students.

Today's science will set the agenda for tomorrow's socio-economic activities. It is, therefore, extremely important to spread the message of science, its unprecedented possibilities and awareness on issues in society. CCMB is committed to this cause: we organize a number of activities under which the CCMB community interacts with young students and the public from across the country every year through its open day programme, educational tours, visits of police, forest, judiciary and industry officials.

A changing set of technologies, and newly emerging possibilities & challenges keep laboratories such as CSIR-CCMB at the centre stage where we are expected to deliver to the needs and aspirations of the nation. I have no doubt that CCMB is ready to meet this challenge.

Rakesh Kumar Mishra
Director

The background features a faint, light-colored ribbon diagram of a protein structure. In the center, there are two decorative swooshes, one on the left and one on the right, each with a yellow outer edge and a dark blue inner edge. The text "I. Charter" is centered between these swooshes.

I. Charter

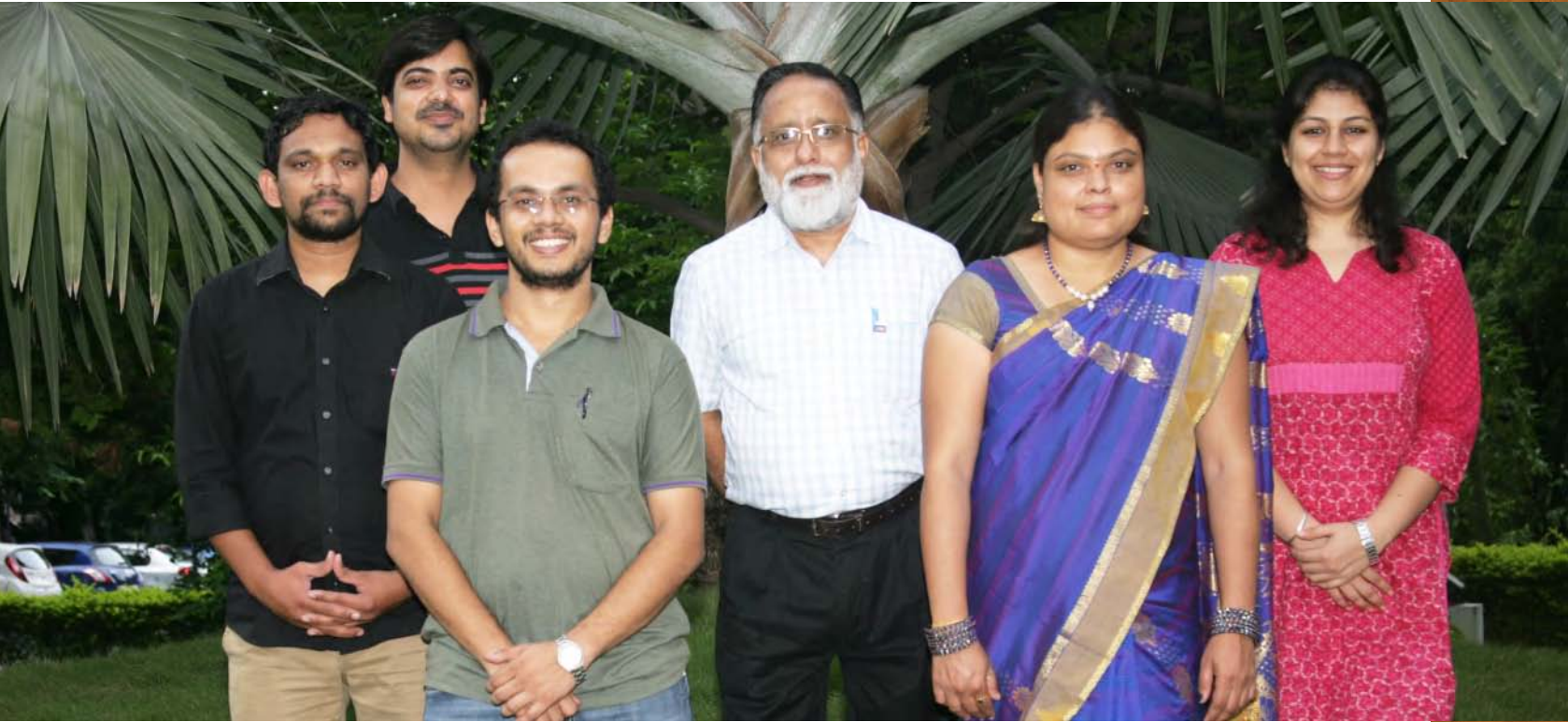
The Centre for Cellular and Molecular Biology (CCMB) is one of the constituent national laboratories of the Council of Scientific and Industrial Research (CSIR), New Delhi, the premier multi-disciplinary research organization in the country funded by the Government of India. It was set up formally in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

Ramesh Kumar Aggarwal

Molecular Genetics and Developmental Biology



From Left to Right: Purushotham, Lomous Kumar, Harsh Joshi, Ramesh K. Aggarwal, Arcot K. Nagamani, Chhavi Dawar

RESEARCH INTERESTS:

- Epigenetics of temperature-dependent sex determination
- DNA Markers: development and applications in crop improvement
- Barcoding of Anurans of India
- Evolution/phylogeography of endangered species
- Decoding of Indian genomes

Our group has identified several candidate genes having putative role in TSD, described new species of anurans, developed >2500 species specific genic-/genomic microsatellite markers, constructed the first-generation framework molecular linkage maps of coffee and mulberry, established that the Indian wolf populations represent new species, i.e., *Canis indica* and *Canis himalayensis*, discovered many new frog species and showed that the olive ridleys in Indian waters are the ancient source population for the olives in other global basins.

Selected recent publications

1. Jegath Janani S, Vasudevan Karthikeyan, Prendini Elizabeth, Dutta Sushil Kumar, and Aggarwal Ramesh K (2017). A new species of the genus *Nasikabatrachus* (Anura, Nasikabatrachi-dae) from the eastern slopes of the Western Ghats, India. *Alytes* 34(1-4), 1-19. (the paper is covered extensively by National/ international press/online news groups, including ones like National Geography).
2. Aggarwal RK, Dawar C, Phanindranath R, Mutnuri L and Dayal AM. (2016). Draft genome sequence of a versatile hydrocarbon-degrading bacterium, *Rhodococcus pyridinivorans* strain KG-16, collected from oil fields in India. *Genome Announcement* 4(1): e01704-15.
3. Chandramouli SR, Vasudevan K, Harikrishnan S, Dutta SK, Janani SJ, Sharma R, Das I and Aggarwal RK. (2016). A new genus and species of arboreal toad with phytotelmonous larvae, from the Andaman Islands, India (Lissamphibia, Anura, Bufonidae). *ZooKeys* 555: 57-90.
4. Prakash N S, Jeena Devasia, Ramesh K Aggarwal, and Raghuramulu Y (2017) COFFEE. In: Biotechnology of Plantation Crops, Eds: Chowdappa P, Anitha Karun, Rajesh M K, Ramesh S V, Chapter 17, pp 355 - 404, (ISBN: 978-93-5124-836-1) *Daya Publishing House*, New Delhi.

Our main research interest is to understand the *eco-devo* of temperature-dependent sex determination (TSD) using Indian mugger as a model system. We are equally interested in application oriented studies involving development/utilization of genomic resources useful for crop improvement, wildlife conservation and disease diagnostics. Lately, we have embarked on whole genome sequencing of Indian mugger, apple, mulberry and a few interesting microbial isolates of clinical/applied significance.

Temperature-dependent sex-determination: Sex-determination is one of the most important developmental decisions occurring early in embryogenesis and is central to the existence/survival of a species. Broadly there exist two mechanisms of sex determination in vertebrates: Genetic Sex Determination (GSD) that involves specific gene(s) and/or highly specialized sex chromosomes, and Environmental Sex Determination (ESD), wherein simple environmental cues appear to be the primary determinants of the sex. Temperature dependent Sex Determination (TSD) is the most common ESD mechanism seen in many reptilians. Indian mugger (*Crocodylus palustris*) is one such species and thus, provides an ideal system to study the role of epigenetic factors such as environmental temperature in vertebrate development, more specifically, in sex-determination.

We have been using Indian mugger as an experimental system to identify the putative genetic mechanism(s) underlying TSD. We have isolated and characterized the crocodile homologues of a few of the evolutionarily conserved, sex-related genes, and have also identified a number of novel candidate genes that show differential expression at Male-/Female-Promoting Temperature (MPT/FPT) in the bipotential gonads. Our studies on *cpSox9* and *cpDmrt1*, the crocodile homologues of *Sox9* (Sry-related transcription factor *Sox9*) and *Dmrt1* (doublesex-/mab-3 related transcription factor-1), as well as a few other candidate genes demonstrate that generation of sex-specific/unique mRNA transcripts by 'extensive alternate splicing', is the norm of the complex genetic interplay underlying the molecular basis of TSD. More recently, we have identified/isolated several isoforms of heat-shock proteins (*cp_HSPs*) and heat-shock factors (*cp_HSF1* and related genes) that may be involved in sensing the temperature stimuli to initiate the set of genetic factors during TSD. Interestingly, some of these isoforms also seem to have sex-specific differential expression through Temperature-Sensitive Window (TSW) in the GAM complex in our preliminary studies.

To expedite our efforts to identify the putative candidate gene(s) underlying the TSD, we initiated whole/global transcriptome profiling of GAM and brain tissues of male/female embryos using the high-throughput NGS approaches. We have now generated NGS data for 36 GAM and brain transcriptomes (ca. 6.0 GB cleaned sequencing data/transcriptome), representing biological duplicates of developmental stages through TSW (stage 21 to 25th) using Illumina *HiSeq* and Roche-454 platforms. The clean sequence reads are normalized to construct a reference data set that is being used to assemble/annotate *denovo* sex-/stage-specific GAM transcriptomes, followed by comparative analysis to identify/characterize differentially expressed genes. Primary *denovo* reference assemblies have been completed with Trinity, a de-bruin based assembler working on fixed k-mer value of 25 giving an average N50 value of 2.6Kb. Presently we are optimizing the k-mer value to be used for multi-kmer multi tool approach to obtain more complete assemblies using various assemblers like Oases, SOAP-denovoTrans and TransAByss. Simultaneously, we have also initiated Whole-Genome Bisulphite Sequencing (WGBS) using Illumina platform to ascertain the methylation patterns of the gene space (especially regulatory regions/ promoter sequences of selected candidate genes) in the genomic DNA of GAM tissues; this is expected to help us understand the epigenetic control (if any) of gene(s) expression that may underlie the TSD.

Development and application of DNA markers: DNA markers provide high genetic resolution and have revolutionized genetic analysis for enumeration, management, utilization of biodiversity resources, understanding origin, evolution, phylogeography of extant species, or genetic enhancement of existing agriculturally important variety/cultivars. Development and application of DNA marker tools have been a major focus of our research, which is carried out in collaboration with various national and international institutes.

Germplasm characterization and linkage studies in rice, coffee, mulberry and apple: Our earlier studies on rice germplasm including the wild rice species and their derivatives have helped us assign two new genomes (GG, HHJJ) of *Oryza*, provided empirical data to support Gondwanaland as the centre of origin for *Oryza*, identified novel potential rice sources for early nodulin gene homologues, and resulted in a DNA polymorphism database useful for the identification, protection and improvement of elite Basmati and specialty rice varieties of India.

Our other more recent major interest has been to create genetic and genomic resources for orphan tree crop species of socio-economic significance. During the last few years, we have developed: a) large repertoires of genomic and genic microsatellite markers of coffee, mulberry and apple; b) molecular linkage maps of robusta coffee and mulberry; c) leaf-specific EST resources of coffee and mulberry. Similarly, we have identified a number of Resistance Gene Analogues in secondary gene pool of coffee, and potential germplasm sources of resistance to powdery mildew in mulberry, which are expected to be useful in breeding for disease-resistance germplasm in these crops. We have also developed/established a pseudo-testcross population of apple from a cross of two elite varieties, i.e., 'Red Delicious' and 'Maharaji', in collaboration with our partners from Jammu University, Kashmir University, YSPUF&H, and GBPIHED. This is the first such genetic resource developed in the country, and will greatly facilitate linkage mapping and QTL studies for this important fruit crop. Our similar work on mulberry, being carried out in collaboration with CSRTI, Berhampore (WB) has resulted in identification of a number of putative QTLs for agronomically important traits, as well as, identification of a few High-yielding/resistant progenies from the mapping population (which have the potential to be developed directly in HYV suited to West Bengal environment).

Wildlife conservation studies: We have been interested in genetic characterization of extant populations of endangered species, and to enable the same, we have developed species-specific microsatellite markers for many species, viz., Indian mugger, red panda, olive ridley turtles and a number of frog species. Our work has given new perspective to the evolution and phylogeography of wolves, anurans, olive ridleys and crocodylians in

the Indian subcontinent, and highlights the need for conservation of their fragile environment. Significantly, we have shown that: a) the Indian wolf populations represent two new species (*Canis indica* and *Canis himalayensis*) basal to the grey wolf-dog lineage, b) anuran endemism in the Western Ghats and other Gondwana break-up landmasses is much earlier than the CT boundary; c) the olive ridley in Indian waters are the source ancestral population for ridleys found across other global basins; and d) the two gharial genera (*Gavialis* and *Tomistoma*) are closer to each other, and together with crocodylids represent a distinct genetic lineage that has diverged from that of alligators and caimans during cretaceous era about 80 - 100 MYA.

During the recent years, we have been actively involved in documenting the anuran diversity across "Biodiversity Hotspots" of the country. We have now generated barcode signatures for ca. 520 individuals representing >40 anuran species of 20 genera; each barcode signature comprises of ca. 2000 bp DNA sequence spanning four different mtDNA domains. Preliminary analysis of the barcode data have indicated many new candidate species/cryptic species (which are yet to be described). We have now described one such new species (Figure 1a,b) that we have named *Nasikabatrachus bhupathi*, in the memory of one of our friend, a young and dynamic herpetologist of India Dr. S. Bhupathy, who lost his life in one of the field expedition last year. The new frog is a significant discovery as it represents the only second member of an enigmatic unique anuran family *Nasikabatrachidae* that was erected a few years back based on another frog find i.e. pignose frog from India by us and another group.

Sequencing of Indian genomes: As part of the initiative under 12 FYP projects of CSIR, we have initiated efforts towards *de novo* sequencing of the



Fig.1:(a) Dorsolateral view of the Holotype of *Nasikabatrachus bhupathi* sp. nov.

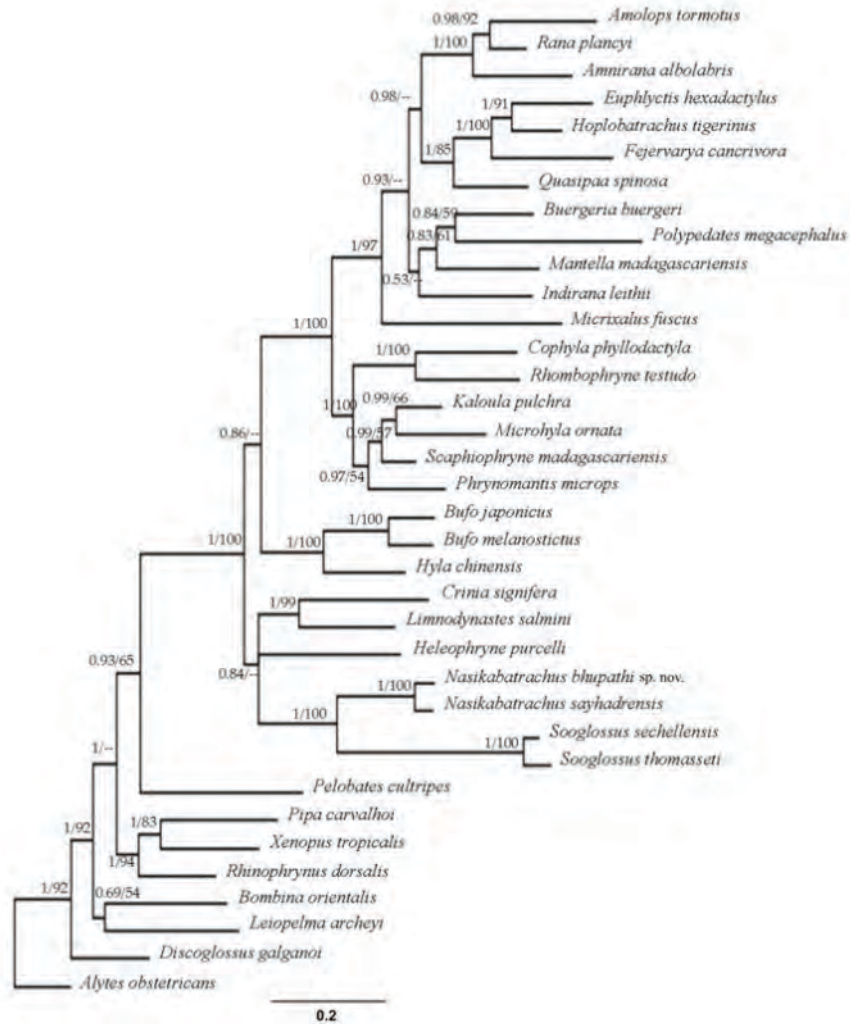


Fig.1: (b) a Bayesian phylogenetic tree inferred from concatenated two partial mitochondrial genes (12S rRNA and 16S rRNA) showing the new frog as the congener of the *N. sahyadrensis* (support values of the nodes are written above each node: Bayesian posterior probabilities, followed by ML bootstrap values).

whole genome of Indian mugger. For the purpose, we are sequencing an alpha male mugger 'Makara' that had been 'the prime sire in breeding programs at the Crocodile Bank, Chennai in 1970s/1980s. We are attempting 'Hybrid sequencing approach' using three different NGS platforms (Roche-454, PacBIO, and Illumina *HighSeq/MiSeq*) that significantly differ in the size and depth of the sequencing read data. To date, we have generated ca. 389 Gb raw data using multiple libraries and HiSeq/MiSeq chemistry on the Illumina sequencer. The data have been quality trimmed and cleaned for noise, and is being used to standardize the various parameters (like k-mer, window-size, bubble size etc) and different assemblers (like SOAP denovo, velvet,

Masurca, Platanus, Discover) to generate an optimal *de novo* genome assembly of Indian mugger. Presently, we have initiated efforts to generate long-read data providing ca. 50X genome coverage using the PacBio chemistry; availability of which is expected to help obtain an robust high-quality *de novo* draft genome of Indian mugger. Simultaneously, we are trying to sequence the genomes of apple and Indian mulberry. We have now generated considerable raw sequencing data (~150 -200 Gb for each plant species). The plant WGS data will be used to extract, assemble and annotate respective mitochondrial and chloroplast genomes and also *de novo* whole genome assemblies. These genomes will add to our efforts to investigate complex important traits in these plants and development of DNA based markers.

Amit Asthana

Applications of Microfluidics, Micro and Nanotechnology in Life Sciences



From left to right (back row): Ira Bhatnagar, Shahila Parween (Front row): O Gopi Suresh, Amit Asthana, Saurabh K. Srivastava

RESEARCH INTERESTS:

- Non-conventional methods to fabricate microfluidic devices.
- Microfluidic device for proteomic analysis to understand wound healing mechanisms
- Point-of-care diagnostic devices based on paper-microfluidics
- Paper-based devices as Raman immune-sensor
- Biopolymer microfluidic devices for tissue engineering and cell culture
- 3D printing and 3D cell culture
- Generation of “site targeted” drug delivery vectors for drug delivery and diagnosis, using microfluidic device

Our group uses micro and nanotechnology to address biological problems. Using a non-conventional method developed in our group to fabricate microfluidic devices, termed as “modified embedded templates”, we have recently demonstrated an approach to generate hollow alginate microfibrils with controlled wall thickness and inner diameter using the above mentioned microdevices. These devices are also used to generate PLGA and alginate microparticles in the group.

Selected recent publications

1. Bhatnagar I, Mani Vasagan P and Bramhachari PV (2016). Opening Avenues in Marine Probiotics – Present and Future. *In: Nolle LML (Ed.), Marine Microorganisms: Extraction and Analysis of Bioactive Compounds.* CRC Press, Boca Raton.
2. Bramhachari PV, Mutyala S, Bhatnagar I, Pallela R (2016). Novel Insights on the Symbiotic Interactions of Marine Sponge-Associated Microorganisms: Marine Microbial Biotechnology Perspective. *In: Pallela R, Ehrlich H (Eds.). Marine Sponges: Chemicobiological and Biomedical Applications,* Springer-Verlag, Germany, pp. 69–95.
3. Bhatnagar I, Pallela R, Bramhachari PV and Ealla KKR. (2016). Chronicles of Sponge Biomaterials: The Saga in Biomedicine. *In: Pallela R, Ehrlich H (Eds.). Marine Sponges: Chemicobiological and Biomedical Applications,* Springer-Verlag, Germany, pp.315-327.
4. Pham UHT, Hanif M, Asthana A and 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.
5. Micheal I J, Aditya J, Vidyasagar, Kiran Kumar B, Naveen Kumar M, Amit Asthana and Ch Mohan Rao (2014) Foil assisted replica molding for fabrication of microfluidic devices and their application *in vitro*, *Lab-on-a-chip* 14 (19): 3695–3699.

As a part of our attempts to fabricate affordable diagnostic devices, we are currently exploring non-conventional ways to fabricate polymer- as well as paper-based microfluidic devices. Among all the affordable devices mentioned in the literature, paper-based microfluidics is considered a potential “game-changer” in the field of diagnostics because of the affordable prices of paper as a substrate. In our group, we have developed a “truly single step” method of fabricating paper devices using an ink developed in CCMB and permanent ink pen. Currently we are using 4 different ways of making devices (1) wax printer (2) XY plotter with permanent ink pen (3) XY plotter with ink developed in our lab (4) laser cutter. Among the various applications of paper based devices, a few are listed below:

Cost effective and efficient paper-based viscometer (with Dr. Ch Mohan Rao)

The use of paper-based devices for affordable diagnostics is gaining interest due to unique advantages like affordability, portability, easy disposability and inherent capillarity. As capillary transportation is an integral component of paper-based devices, a low sample volume with faster measurement becomes an additional advantage. We have exploited the aforementioned features of paper-based devices to develop a simple microfluidic device suitable for measuring viscosity of Newtonian fluids as well as a few non-Newtonian fluids. The paper fabricated for viscosity measurements has been designed with arms that support a test channel (Figure 1) and also helps in overcoming the issue of deformation that is usually caused due to the flow and

wetting of the paper substrate. With an excellent correlation coefficient of 0.9997 when compared to the Ostwald’s viscometer, this paper-based device could serve as a better alternative to conventional methods for viscosity measurements. The major advantage that a paper-based viscometer offers over a conventional viscometer includes its time efficiency, cost effectiveness, and low sample volume requirement. Moreover, these paper-based devices can also be effectively used to estimate molecular weights of polymers with reasonable accuracy. The molecular weight of a model polymer, i.e. Dextran T500, is ~500000 and with our paper-based devices we have achieved an estimated molecular weight of 496085 by plotting reduced and inherent viscosities. The point of intersection of both the lines (reduced and inherent viscosity) at the Y-axis gives the intrinsic viscosity (η). The value of intrinsic viscosity along with empirical parameters characteristic of a particular solute-solvent pair when used in Mark-Houwink equation gives the molecular wt. of a polymer. Further with our paper-based viscometer one can rapidly ascertain the quality of proteins. We have used BSA and Lysozyme as model proteins and have studied the viscosity of native and denatured proteins (achieved by heating). The viscosity values of native and denatured proteins were compared and a significant increase in viscosity was observed in case of denatured proteins. Folded globular proteins are compact, however when they are unfolded (denatured) the chains extend and alter the viscosity of the solution. Therefore we can easily use a paper based viscometer to ascertain protein stability and purity.

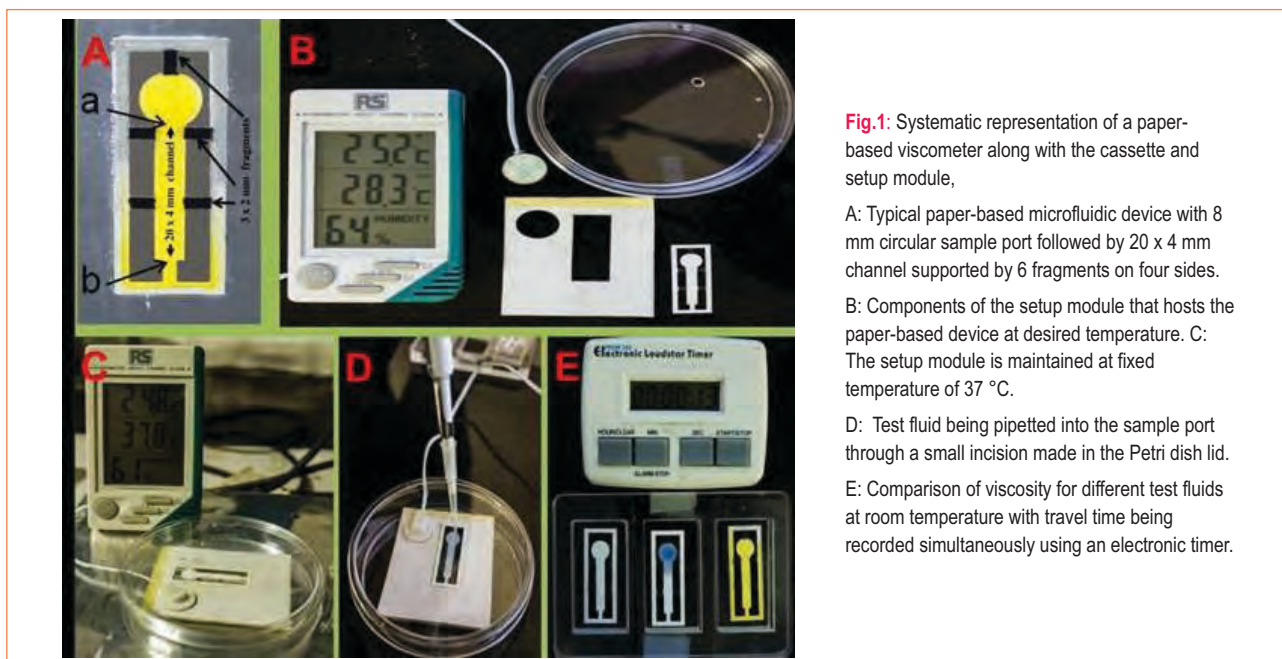


Fig.1: Systematic representation of a paper-based viscometer along with the cassette and setup module,
 A: Typical paper-based microfluidic device with 8 mm circular sample port followed by 20 x 4 mm channel supported by 6 fragments on four sides.
 B: Components of the setup module that hosts the paper-based device at desired temperature. C: The setup module is maintained at fixed temperature of 37 °C.
 D: Test fluid being pipetted into the sample port through a small incision made in the Petri dish lid.
 E: Comparison of viscosity for different test fluids at room temperature with travel time being recorded simultaneously using an electronic timer.

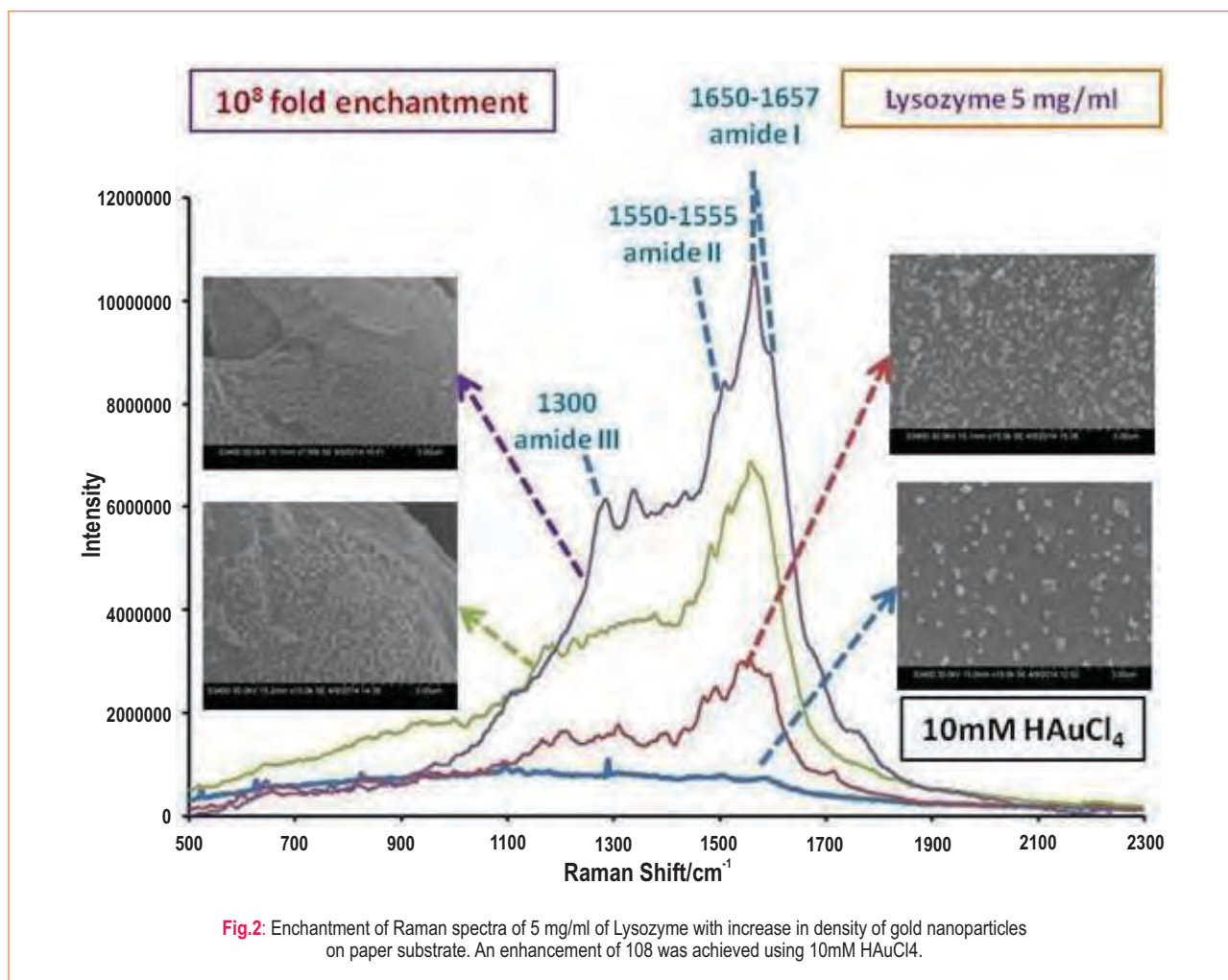


Fig.2: Enchantment of Raman spectra of 5 mg/ml of Lysozyme with increase in density of gold nanoparticles on paper substrate. An enhancement of 10⁸ was achieved using 10mM HAuCl₄.

Paper-based Surface Enhanced Raman microscopy substrates for detection enhancement

Raman spectroscopy is a technique that measures the shift in wavelengths of light arising due to inelastic scattering. It is used to characterize the vibrational and rotational state of a molecule and is a signature of a particular molecule. However, typical Raman signals are too weak to be detected and require some sort of augmentation. Surface-Enhanced Raman Spectroscopy (SERS) is a modification of Raman spectroscopy where the analyte to be investigated is layered on a metallic surface to enhance the Raman signal. The

metallic surface has to be uneven for better results. Hence, either a roughened surface or substances with small radii of curvature are used. Metallic nanoparticles made of silver or gold are an excellent substratum for SERS. In our lab we have created four different ways of fabricating rapid and affordable paper-based SERS substrate for potential clinical applications. Lysozyme and Rhodamine 6G were used as model molecules to evaluate SERS performance of the as-synthesized paper-based SERS substrates. Figure 2 shows some of the SEM pictures of paper devices and Raman spectra of lysozyme showing enhancement in the Raman signals.

A S Sreedhar

Stress Biology and Molecular Medicine



From left to right: Siripini Satish, Pankaj Kumar, Hatim Kara, Vykuntham Naga Gowthami, Amere Subbarao Sreedhar, Khanderao Paithankar, Guntipally Mounika, A Vijaya Lakshmi; **Inset-left:** Kanugovi Abhijnya Vijayavittal, **inset-right:** Akhil Kotwal

RESEARCH INTERESTS:

- Enforced senescence as a tumor suppressor mechanism
- Hsp90 in the epigenetic regulation of cancer
- Hsp90 in the cross-talk between acquired multidrug resistance and metastasis of cancer cells
- Evaluation of mitochondrial chaperone, Trap1 role in cancer cells

Our group

- identified tumor selective functions of Hsp90
- demonstrated that enforced senescence through Hsp90 inhibition acts as tumor suppressor mechanism
- identified novel roles of Hsp90 in multidrug resistance
- identified potential role of Trap1 in mitochondrial dynamics and metabolism

Selected recent publications

1. Kanugovi AV, Joseph C, Sreedhar AS (2016) The tumor suppressor p16^{INK4a} expression bypasses 17AAG mediated cellular effects in human neuroblastoma, IMR-32. *Transl Med.* (6) 74.
2. Kanugovi AV, Sugunan S, Joseph C and Amere SS (2015). The prosurvival activity of HSF2 in response to autophagic stress is triggered by HSF2-dependent transactivation of BTG2. *Mol Biol Cell.* 26(25):4523.
3. Kanugovi AV and Amere SS (2014). Oncogene de-addiction from Hsp90 induces senescence-like phenotype in malignant and metastatic tumor cells. *Mol. Biol. Cell.* 25(25):3987.
4. Sarangi U, Singh MK, Abhijnya KVV, PrasannaAnjaneya Reddy L, Siva Prasad B, Pitke VV, Paithankar KR and 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)35-5.
5. Sarangi U, Paithankar KR, Ujwal Kumar J, Subramaniam V and Sreedhar AS(2012). 17AAG treatment accelerates doxorubicin induced cellular senescence: Hsp90 interferes with enforced senescence of tumor cells. *Drug Target Insights*, 6: 19-35.

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 oncogene addiction

We earlier demonstrated that mutated oncogenic Raf-1 (CAAX-Raf) gets addicted to Hsp90. As a result, the conformation specific Hsp90 inhibitor 17AAG, destabilizes Raf-1 and promotes cellular senescence. It is proposed that Hsp90-kinase interaction occurs through a common surface on kinases, the $\alpha 5\beta 4$ loop. Through co-precipitation experiments in vitro, we observed CAAX-Raf interacting with Hsp90 α more efficiently than Hsp90 β . The co-chaperone, cdc37 was also found to be pulled down in the complex. Subsequently using Hsp90 deletion constructs and CAAX-Raf, we observed wild type Raf interacting with Hsp90 without the hinge region (a region between N-terminus and the middle domain), but not CAAX-Raf, indicating that the hinge region of Hsp90 is responsible for CAAX-Raf interaction. These results indicated that oncogene addiction to Hsp90 occurs through kinase interaction with the hinge region, but not through the $\alpha 5\beta 4$ loop.

Enforced senescence as a tumor suppressor mechanism

Earlier we demonstrated that Raf or Ras addiction to Hsp90 sensitizes cells to Hsp90 inhibition, however, this induces senescence by activating the DNA damage response (DDR) pathway mediated through p53-p21WAF-1 axis. To demonstrate tumor suppression in vivo, we used the mouse xenograft model as a tool. Since oncogene de-addiction from Hsp90 is resulting in the activation of senescence program, we believe that activation of senescence-like phenotype may have clinical advantage. To further evaluate our findings in vivo, nude mice were subcutaneously injected with wild type and CAAX-Raf transfected cells, treated with 17AAG, and solid tumor growth was monitored over a period of time. Compared to wild type Raf cells, CAAX-Raf cells showed increase in tumor growth and upon 17AAG treatment were unable to develop tumors. The significant decrease in tumor volume and cells at tumor site of injection indicates antitumor effects of 17AAG in CAAX-Raf cells. The increase in

senescence associated β -galactosidase activity in tumor cells only in response to the combination treatment suggests that the lack of tumorigenic potential is due to activation of a senescence program. Subsequently CAAX-Raf transfected cells were injected intravenously and found that the tumor is being metastasizing to lungs. However i.v injections followed by 17AAG treatment resulted in decrease in metastasizing effects of the tumor to lungs. Our results demonstrate that oncogene de-addiction to Hsp90 activates the senescence program thus acting as a tumor suppressor mechanism.

Hsp90 in the epigenetic regulation of cancer

Cancer emerges from both genetic and epigenetic mechanisms. Earlier we showed the involvement of Hsp90 in the epigenetic regulation of Rb and Raf-1. We hypothesized that Hsp90 regulates Rb transcription at least by three mechanisms, (1) through stabilizing CDK4/6, (2) by directly binding to the Rb promoter (3) and through E2F1. Although, the role of Hsp90 in the cytoplasm is well known through its stabilization of protein kinase clients, its nuclear functions are less studied. Our study aims at understanding novel interactions and functions of nuclear Hsp90. We found that E2F1 and E2F2 are novel interacting partners of Hsp90 in the nucleus. As of now, we could explore the role of Hsp90 in the functional stabilization and transcriptional activation of E2F1. Interestingly, we also observed acetylated Hsp90 in the nuclear localization and interaction with E2F1 and E2F2. We are evaluating role of Hsp90 in cell cycle regulation through different E2F transcription factors.

Cancer EMT

Earlier we reported that hypoxia induces EMT in MCF7 and MDAMB-231 breast cancer cells. Interestingly, challenging these cells with chemotherapeutic agents including Hsp90 inhibitors also showed enhanced EMT. While the mechanism of Hsp90 inhibition mediated EMT is not known, the data provided insights that Hsp90 interferes with (or keeps under check) EMT. We asked for the possible mechanism involved in this EMT. Though we did not observe a prominent decrease in E-cadherin expression at the transcription level, we observed a decrease in surface E-cadherin correlating with EMT progression. Since loss of E-cadherin facilitates the translocation of β -catenin to the nucleus to induce the transcription of genes responsible for EMT, we examined its translocation and observed that induced EMT is not dependent on β -catenin. We are now examining the mechanism(s) of therapeutically

induced EMT and its link with Hsp90. Our *in vivo* data indicated decreased metastasis with Hsp90 inhibition in MCF7, and in contrast, we observed increased metastasis in MDAMB-231 cells. We correlated these findings with *in vitro* data and postulated the hypothesis that the amount of stem cell pool in the tumor population decides the fate of cells in response to treatments. MCF-7 cells showed enhanced pluripotency on the onset of EMT and showed sensitivity to Hsp90 inhibition. In contrast, MDAMB-231 cells showed decreased pluripotency, but enhanced stemness leading to enhanced metastasis. We are yet to re-confirm the data with our *in vivo* findings to draw a conclusion.

Hsp90 inhibition and multidrug resistance

Earlier we reported a positive correlation between Hsp90 expression with increased multidrug resistance of cancer cells. Multidrug efflux pumps are located at the plasma membrane and are more specially enriched within the cholesterol rich membrane micro-domains called lipid rafts. We demonstrated co-localization of Hsp90 and p-glycoprotein with the help of dual immunofluorescence. Now we show that an increase in Hsp90 increases the cholesterol accumulation to the lipid rafts that incidentally correlated with the accumulation of Hsp90 itself along with the p-glycoprotein. Further, increased Hsp90 also correlated with increased HMGCoA biosynthesis indicating its possible regulatory role in cholesterol biosynthesis. However, although the HMGCoA inhibitor lovastatin decreased cholesterol biosynthesis, its accumulation at the lipid rafts was not affected, indicating that Hsp90 is majorly involved in cholesterol transport to the lipid rafts. Depletion of cholesterol using methyl β -cyclodextrin (M β CD) decreased both Hsp90 and p-glycoprotein accumulation at the lipid rafts. From these results we demonstrate that Hsp90 potentiates drug efflux activity through enhanced cholesterol transport to the lipid rafts.

Earlier we reported that Hsp90 inhibition increases MMP7 expression and decreases MMP11 expression. We also showed that increased drug resistance may be a consequence of EMT induced by 17AAG. To understand MMP7 and MMP11 mediated metastatic potential of multidrug resistant cells, we examined the *in vivo* tumorigenic potential of MMP7 and MMP11 over expressing cells. From the preliminary results, we found that both cell types show tumor growth but differ in their tumor forming ability. From these results, we learn that MMP7 and MMP11 cells have opposite functions in drug efflux activity as well as in tumor forming ability. These results need to be further examined.

Tumor selective functions of Trap1

Trap1 (TNF receptor associated protein 1) is a nuclear encoded, mitochondrial chaperone belonging to the HSP90 family. Unlike Hsp90, Trap1 functions are not fully understood. Earlier we showed that Trap1 expression increases with the aggressiveness of tumors and is associated with enhanced mitochondrial fission, whereas its knockdown promotes mitochondria fusion. Since mitochondria are central to cellular energy metabolism, we examined the role of Trap1 in regulating mitochondrial metabolism. While measuring the oxygen consumption rate (OCR), we discovered that Trap1 over expression decreases OCR, while its knockdown increases the same. Since altered OCR may alter ATP, we measured ATP and found out that ATP levels showed opposite effect with OCR indicating that ATP production in these cells may be OXPHOS-independent. Further, we also observed that decreased Trap1 itself elicits a hypoxic response. Although, we did not find a direct correlation of Trap1 with mitochondria metabolism, we obtained a role for Trap1 in metabolic reprogramming. We are currently examining the role of Trap1 in mitochondrial dynamics and its impact on metabolic reprogramming.

Purnima Bhargava

Epigenetic Mechanisms of Gene Regulation



Ashutosh Shukla, Purnima Bhargava

RESEARCH INTERESTS:

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

Our group showed that Yeast prefoldin protein, Bud27 regulates the biogenesis of two of the core subunits and hence the assembly of the 17-subunit yeast RNA polymerase (pol) III.

Yeast Paf1 complex has a protective role on damage-prone pol III-transcribed gene which show high transcription activity *in vivo*.

Spt16 subunit of the yeast histone chaperone FACT maintains the Swr1 and H2A.Z levels in the nucleosome downstream (DS) of the terminator at the 3' gene end.

FACT is specifically enriched at the 3' end of all pol III-transcribed genes.

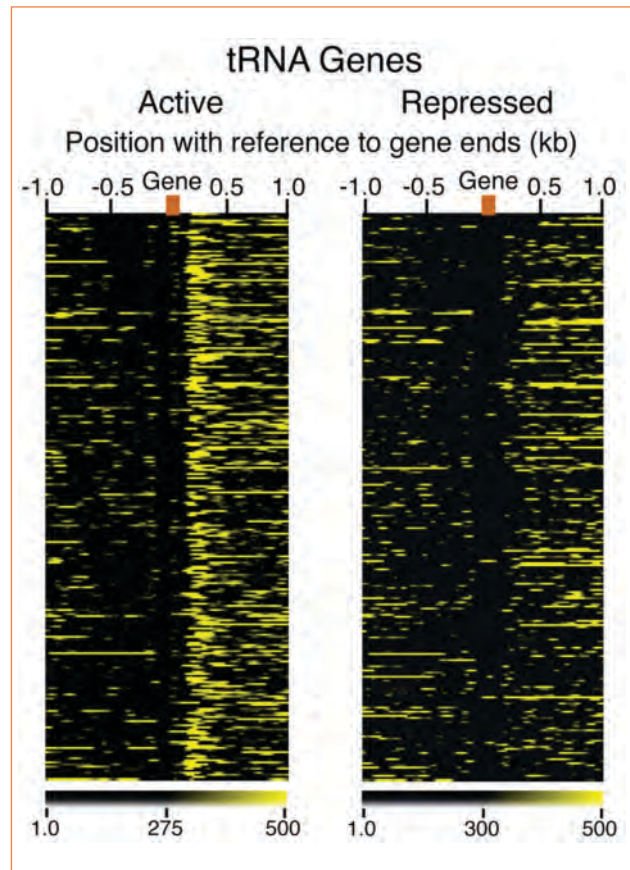
FACT and pol III co-occupy and traverse the gene body together. Pol III delivers FACT to the DS nucleosome at the end of each transcription cycle.

Selected recent publications

1. Belagal P, Normand C, Shukla A, Wang R, Dez C, Bhargava P and Gadal O (2016) Decoding the principles underlying the frequency of association with nucleoli for RNA polymerase III-transcribed genes in budding yeast. *Mol. Biol. Cell.* E16-03-0145.
2. Vernekar D V and Bhargava P (2015) Yeast Bud27 modulates the biogenesis of Rpc128 and Rpc160 subunits and the assembly of RNA polymerase III. *BBA-GRM* 1849, 1340-1353.
3. Albert B, Mathon J, Shukla A, Saad H, Normand C, Legere-Silvestre I, Villa D, Kamgoue A, Mozziconacci J, Wong H, Zimmer C, Bhargava P, Bancaud A and Gadal O (2013) Systematic characterization of the conformation and dynamics of budding yeast chromosome XII. *J Cell Biol.* 202: 201-210.
4. Kumar, Y. and Bhargava, P. (2013) A unique nucleosome arrangement, maintained actively by chromatin remodelers facilitates transcription of yeast tRNA genes. *BMC Genomics* 14:402.
5. Mahapatra, S., Dewari, P.S., Bhardwaj, A. and Bhargava, P. (2011) Yeast H2A.Z, FACT complex and RSC regulate transcription of tRNA gene through differential dynamics of flanking nucleosomes. *Nucl. Acids Res.* 39, 4023-4034.

Eukaryotic transcription by the RNA polymerases takes place in a generally repressive chromatin milieu. Organization of the genome into chromatin restricts the access of transcription machinery to its templates. We have been studying the relationship of chromatin and transcription using yeast as model eukaryotic system. In the budding yeast *Saccharomyces cerevisiae*, ~300 genes 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 TFIIC and TFIIB, which assemble the transcription complex utilizing the intra-genic promoter elements. Generally no upstream regulatory sequences are found and as compared to the genes transcribed by pol II, only 3-5 classical, DNA-binding regulators are known for the pol III-transcribed genes. Recent evidences for regulation by chromatin-related mechanisms have been unraveling novel mechanistic details involved in transcription by pol III. We had earlier reported a unique arrangement of nucleosomes on all pol III-transcribed genes in *S. cerevisiae*. Gene body of all tRNAs was found in a nucleosome-free region (NFR) bordered by two nucleosomes containing the histone variant H2A.Z. The upstream (US) nucleosome is strongly positioned while the downstream (DS) nucleosome is positioned at various distances from the gene terminator. Results also suggested that the downstream nucleosome dynamics provides a novel regulatory mechanism for transcription of most of the pol III-transcribed genes *in vivo*. Further, measurement of pol III occupancy revealed that all genes are actively transcribed in the budding yeast but to different levels. We had shown that a prefoldin molecule Bud27 directly interacts with pol III and regulates the cytoplasmic assembly of the functional pol III as its deletion affects the total levels and ratio of the two largest, core subunits of pol III Rpc160 and Rpc128.

Pol III transcribes mostly house-keeping genes. Yet, 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. During this year, we found that all five subunits of one of the well studied transcription factor of pol II, Paf1 complex, interact with the pol III transcription complex. Paf1 localizes to the pol III target genes and its deletion results in higher pol III transcription and higher pol III occupancy on the genes. A high transcription rate of the genes *in vivo* causes the replication fork stalling at the genes, making them prone to DNA damage. We found that Paf1 is required to counter the toxicity of the genotoxin HU at the pol III-transcribed genes, where it reduces the transcription activity by keeping pol III levels low on the genes.

One of the variants of the histone H2A, H2A.Z has been found associated with the 5' ends of both active and repressed genes. Comparatively lower levels of H2A.Z has been reported in nucleosomes flanking the genes transcribed by pol III. We had earlier proposed that histone chaperone FACT may serve as H2A.Z chaperone on tRNA genes. Our genome-wide occupancy study on the yeast FACT subunit Spt16 revealed overlapping profiles with H2A.Z and the SWR1 complex, in the region

downstream (DS) of the 3'-end of the tRNA genes. H2A.Z is deposited in the nucleosomes by the Swr1 complex while Spt16 evicts H2A.Z. Spt16 maintains Swr1 and H2A.Z levels on these genes. Spt16 also maintains the occupancy and positioning of the DS nucleosome. Deposition of H2A.Z confers instability to the tDNA flanking nucleosomes. Higher H2A.Z levels in the DS than the US nucleosomes may make them more movable and hence suitable for quick response to regulatory cues.

H2A.Z has been proposed to mark the activity status of the genes. We found both H2A.Z and Spt16 are not required for tDNA transcription by pol III. In comparison, on the longer, non-tRNA pol III-transcribed genes, a disruption of gene body nucleosomes by Spt16 facilitates their transcription. Spt16

does not interact with TFIIB but it interacts with TFIIC and pol III physically. Higher Spt16 levels in the DS region are reduced to basal levels under nutrient starvation when pol III is lost from the genes. Active transcription and not merely pol III presence leads to Spt16 enrichment in the DS region. This showed that the DS enrichment of pol III is associated with traversing of the genes by pol III. Spt16 travels with pol III during transcription and pol III delivers Spt16 to the DS nucleosome at the gene terminator with the end of each transcription cycle. Thus, by associating with pol III, Spt16 has a direct approach to sense the transcription status at the pol III-transcribed genes. This may serve to link H2A.Z levels with the DS nucleosome dynamics, which may play a regulatory role in differential transcription of isogenes.

Venkataditya R Chalamcharla

Regulation of Gene Expression by Affecting the Production or Stability of RNA



Venkataditya R Chalamcharla

RESEARCH INTERESTS:

Regulation of gene expression - by affecting the production or stability of RNA is fundamental to cellular growth, development, and adaptation to short- and long-term environmental stress in all organisms. A vast majority of messenger RNAs (mRNA) and long non-coding transcripts (lncRNA) that are linked to human development and disease are regulated during the elongation phase of RNA polymerase II (RNAPII) transcription. However, the precise molecular mechanisms dictating the production and quality-control of RNA during transcription elongation remain unclear.

Using the fission yeast *Schizosaccharomyces pombe* as a model system, my laboratory is committed: 1. to determine the key factors and mechanisms (genetic and epigenetic) that control the movement (pausing and release) of RNAPII to affect RNA production. 2. to elucidate how aberrant RNAs are recognized and degraded co-transcriptionally. We use genetic and biochemical approaches involving “traditional” and high-throughput methods to address these questions.

Selected recent publications

1. Diego Folco H, Venkata R C, Tomoyasu S, Gobi T, Martin Z, Vanivilasini B, Jothy D, Takeshi M and Shiv I S G (2017) Untimely expression of gametogenic genes in vegetative cells causes uniparental disomy. *Nature* 543 (7643): 126-130.
2. Venkata R C, Diego Folco H, Jothy D and Shiv I S G (2015) A conserved factor Dhp1/Rat1/Xrn2 triggers premature transcription termination and nucleates heterochromatin to promote gene silencing. *PNAS* 112(51): 15548-55.
3. Nathan N L, Venkata R C, Francisca R, Sameet M, Martin Z, Vanivilasini B, Jothy D, Nitika T, Soichiro Y, Ming Z and Shiv I S G (2013) Mtr4-like protein coordinates nuclear RNA processing for heterochromatin assembly and for telomere maintenance. *Cell* 155(5) 1061-74.
4. Venkata R C, Joan M C and Marlene Belfort (2010) Nuclear expression of a group II intron is consistent with spliceosomal intron ancestry. *Genes Dev.* 24(8) 827-836.

Giriraj Ratan Chandak

Complex Human Genetic Disorders



From left to right: Front row: Ashutosh Singh Tomar, S Meraj Ahmed, Seema Bhasker, N Suraj Singh, K Radha Mani, Radhika P Ramachandran, K Pujitha, Sumit Paliwal
Back row: B Ramya, Dilip Kumar Yadav, Lovejeet Kaur, Sara Sajjadi, P Ashok, Divya Sri Priyanka T, Giriraj R Chandak, Akshay Dedaniya, Ajay Deepak Verma, V Jyothi, Prachand Issarapu, Ch Mounika, D Vinay, Swati Bayyana, P Sangam

RESEARCH INTERESTS:

- Gene-nutrient interaction and Developmental Origin of Health and Diseases (DOHaD)
- Genetic susceptibility of type 2 diabetes mellitus and related intermediate traits
- Genetic basis of chronic pancreatitis with special reference to tropical calcific pancreatitis

Our group has provided evidence that genetic basis of complex diseases and related intermediate traits in Indians have unique features. Epigenetic regulation of genes involved in key pathways implicated in type 2 diabetes and related intermediate traits including obesity, in response to maternal (micro) nutrients may explain this variability and provide intervention opportunities for alleviation of future risk of these diseases.

Selected recent publications

1. Prachand I, Paliwal S, Bhaskar S, Giriraj R Chandak (2017) Evolution of Phenotypic and Genetic Profile of Tropical Calcific Pancreatitis. *In* 'Special types of chronic pancreatitis'. © Springer Nature Singapore Pte Ltd. and Shanghai Scientific and Technical Publishers. Z.-S. Li et al. (eds.), Chronic Pancreatitis, DOI 10.1007/978-981-10-4515-8_11.
2. Ahmad M, Sinha A, Ghosh S, Kumar V, Davila S, Yajnik CS and Chandak GR (2017). Inclusion of Population-specific Reference Panel from India to the 1000 Genomes Phase 3 Panel Improves Imputation Accuracy. *Sci Rep.* 7(1):6733.
3. Khot VV, Yadav DK, Shrestha S, Kaur L, Sundrani DP, Chavan-Gautam PM, Mehendale SS, Chandak GR, Joshi SR (2017). Hypermethylated CpG sites in the MTR gene promoter in preterm placenta. *Epigenomics* 9(7):985-996.
4. Murali Krishna, Mohan Kumar G, Veena SR, Krishnaveni G V, Kalyanaraman K, Samuel C K, Patsy C, Clive O, John R C, Chandak G R, Dattatray B, Mathew V, Martin P, Caroline F (2016) Birth size, risk factors across life and cognition in late life: protocol of prospective longitudinal follow-up of the MYNAH (MYsore studies of Natal effects on Ageing and Health) cohort. *BMJ Open*; 6: e012552. doi:10.1136/bmjopen-2016-012552.
5. Boulling A, Masson E, Zou W-B, et al. (2017) Identification of a functional enhancer variant within the chronic pancreatitis-associated *SPINK1* c.101A>G (p.Asn34Ser)-containing haplotype. *Hum Mutat.* 38(8):1014-1024.

Based on the established observation that the phenotype and the clinical course of many complex diseases in Indians is different as compared to Europeans, my group has been investigating 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 tropical calcific pancreatitis, type 2 diabetes mellitus and metabolic syndrome. Over last several years, we have provided evidence that genetic basis of the abovementioned disorders has some differences between Indians and Europeans or Americans. Consolidating further on the earlier results, we are attempting to understand the influence of micronutrients in regulating the genetic risk for chronic diseases through epigenetic mechanisms, which also forms the basis of Developmental Origin of Health and Diseases (DOHaD).

Molecular mechanism of B₁₂ mediated programming of diabetes

DOHaD proposes that susceptibility for complex and metabolic diseases including type 2 diabetes (T2D) originates in the intrauterine life by environmental fetal programming. Maternal nutrition and metabolism are major mechanisms by which intrauterine environment programs the health of the offspring. Micronutrient deficiencies during pregnancy and early development have been associated with adverse pregnancy and long-term health outcomes. Epidemiologic studies have established that low birth weight (LBW) or relative thinness at birth and during early childhood is associated with increased risk of cardio vascular diseases (CVD), stroke, adiposity T2D and metabolic syndrome in adult life. Furthermore, studies have shown that prenatal environment and intake of specific nutrient during pregnancy alters the

development and future risks of metabolic diseases in offspring. In addition, several supplementation studies have described the effect of micronutrient on fetal growth. In Pune Maternal Nutrition Study (PMNS), we have earlier established that Indian mothers are small, thin with low body mass index (BMI) and are hyperhomocysteinemic due to low plasma vitamin B₁₂ levels. We also demonstrated that low maternal B₁₂ levels predict LBW and low B₁₂ and high erythrocyte folate predicts higher adiposity and insulin resistance in offspring. In the follow-up study of the children at 6 years of age showed that children born to mothers with lowest B₁₂ and highest folate levels were most insulin resistant compared to those born to mothers with higher B₁₂ and low/normal folate. Although substantial amount of evidence is available which show that B₁₂ and folate imbalance during early development is central to fetal programming for disease in later life, the underlying molecular mechanism is still elusive.

To understand the molecular mechanism of B₁₂ mediated programming of T2D and obesity, we investigated the DNA methylation changes using Methylated DNA Immunoprecipitation sequencing (MeDIP-seq) approach in 6 years old children born to mothers from the 1) lowest decile of B₁₂ and highest decile folate and were most insulin resistant and 2) highest decile of B₁₂ and lowest decile of folate and were least insulin resistant. We identified several differentially methylated regions (DMRs) in several genes associated with type 2 diabetes, related intermediate traits and their regulatory regions. Furthermore, using *in vitro* techniques, we functionally characterized a specific DMR in the peroxisome proliferator-activated receptor delta (*PPAR δ*) locus as an insulator/enhancer blocker element (Figure 1). We observe that when only SV40E was present upstream

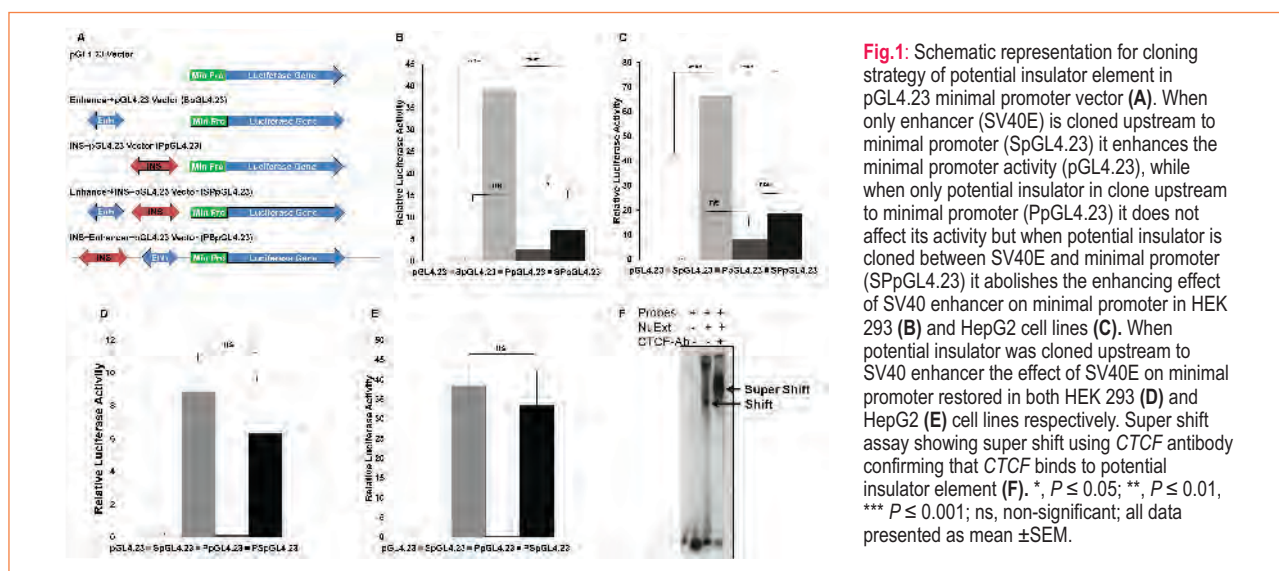
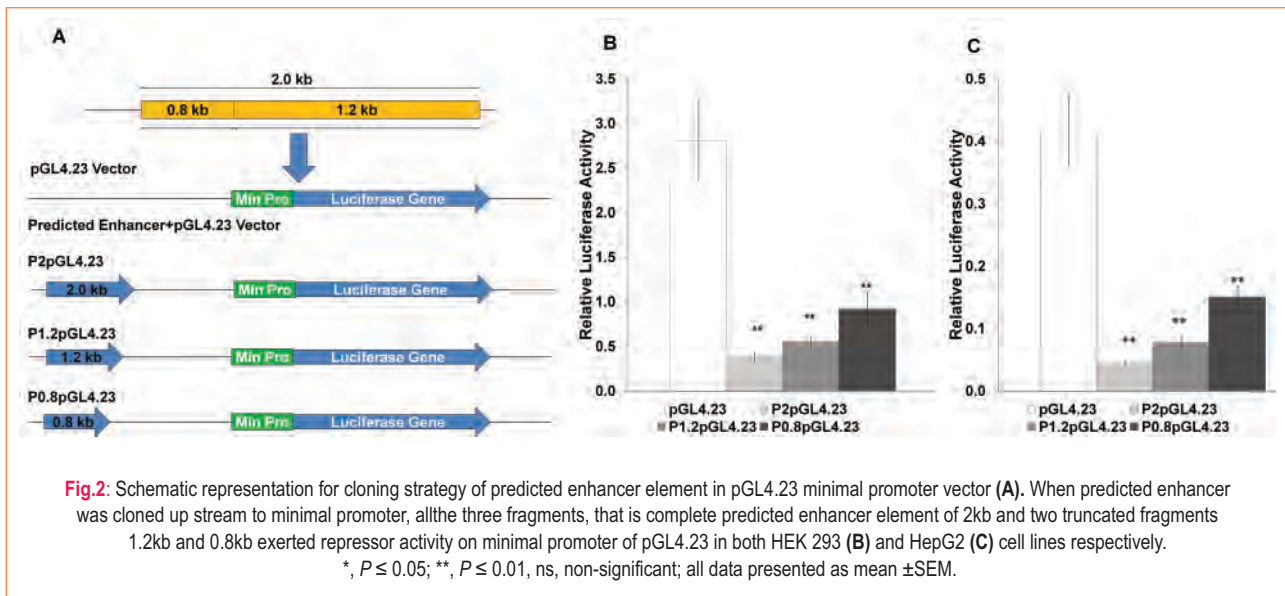
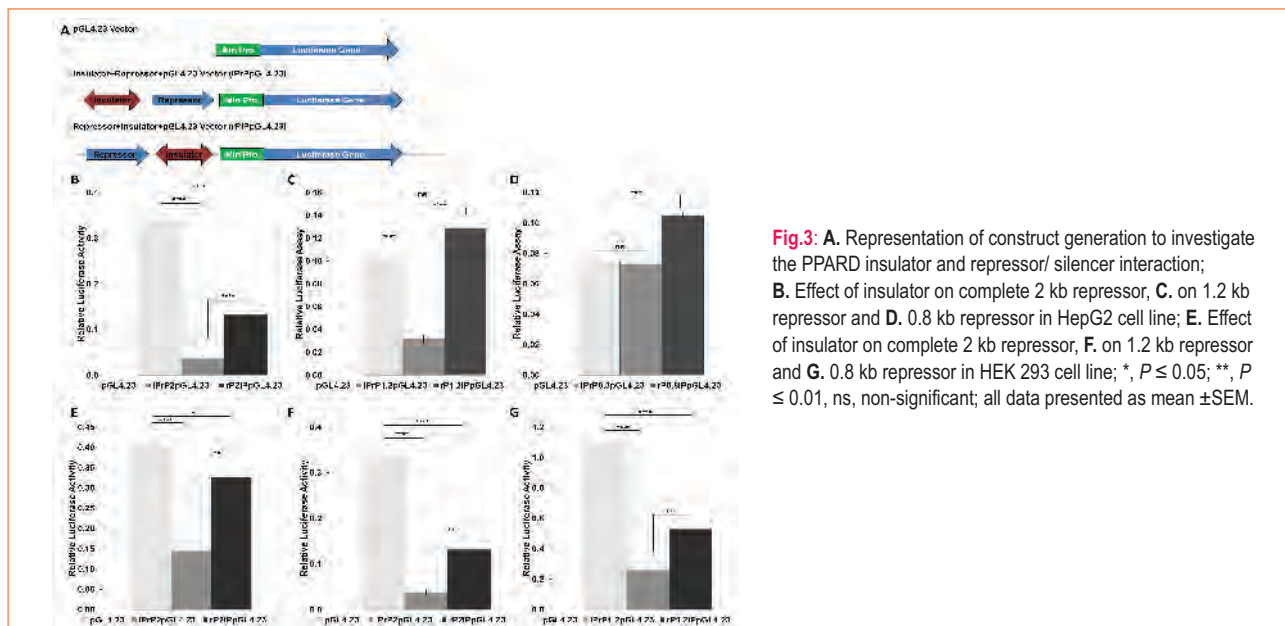


Fig.1: Schematic representation for cloning strategy of potential insulator element in pGL4.23 minimal promoter vector (A). When only enhancer (SV40E) is cloned upstream to minimal promoter (SpGL4.23) it enhances the minimal promoter activity (pGL4.23), while when only potential insulator is clone upstream to minimal promoter (PpGL4.23) it does not affect its activity but when potential insulator is cloned between SV40E and minimal promoter (SPpGL4.23) it abolishes the enhancing effect of SV40 enhancer on minimal promoter in HEK 293 (B) and HepG2 cell lines (C). When potential insulator was cloned upstream to SV40 enhancer the effect of SV40E on minimal promoter restored in both HEK 293 (D) and HepG2 (E) cell lines respectively. Super shift assay showing super shift using CTCF antibody confirming that CTCF binds to potential insulator element (F). *, $P \leq 0.05$; **, $P \leq 0.01$, *** $P \leq 0.001$; ns, non-significant; all data presented as mean \pm SEM.



to the minimal promoter (SpGL4.23), it significantly enhanced the promoter activity of the minimal promoter but no effect on minimal promoter activity was noted when only INS was present upstream to the minimal promoter (PpGL4.23). Interestingly, the effect of enhancer on minimal promoter activity was abolished when INS was present between enhancer and the minimal promoter (SPpGL4.23). We have also characterized a repressor element downstream to the characterized insulator element and demonstrated that when present upstream to the minimal promoter, it suppresses the promoter activity (Figure 2). Next to investigate whether characterized insulator element has potential to inhibit the repressor activity, we have investigated the interaction between them using in vitro techniques. We observed that the silencing

activity of repressor/ silencer remained unaffected when insulator is present upstream to the repressor element, but when present between repressor and minimal promoter, it significantly reduces the effect of repressor/silencer element on the minimal promoter (Figure 3). These observations suggest that abnormal methylation pattern of the insulator element may result in deregulation of PPARD gene which may results in higher adiposity and insulin resistance in children born to mothers with an imbalance of B₁₂ and folate levels in Indians. Since PPARD has an established role in T2D, lipid and glucose metabolism and insulin sensitization, our results aid in understanding the link between B₁₂ mediated risk of T2D and its related intermediate traits such as insulin resistance and adiposity.



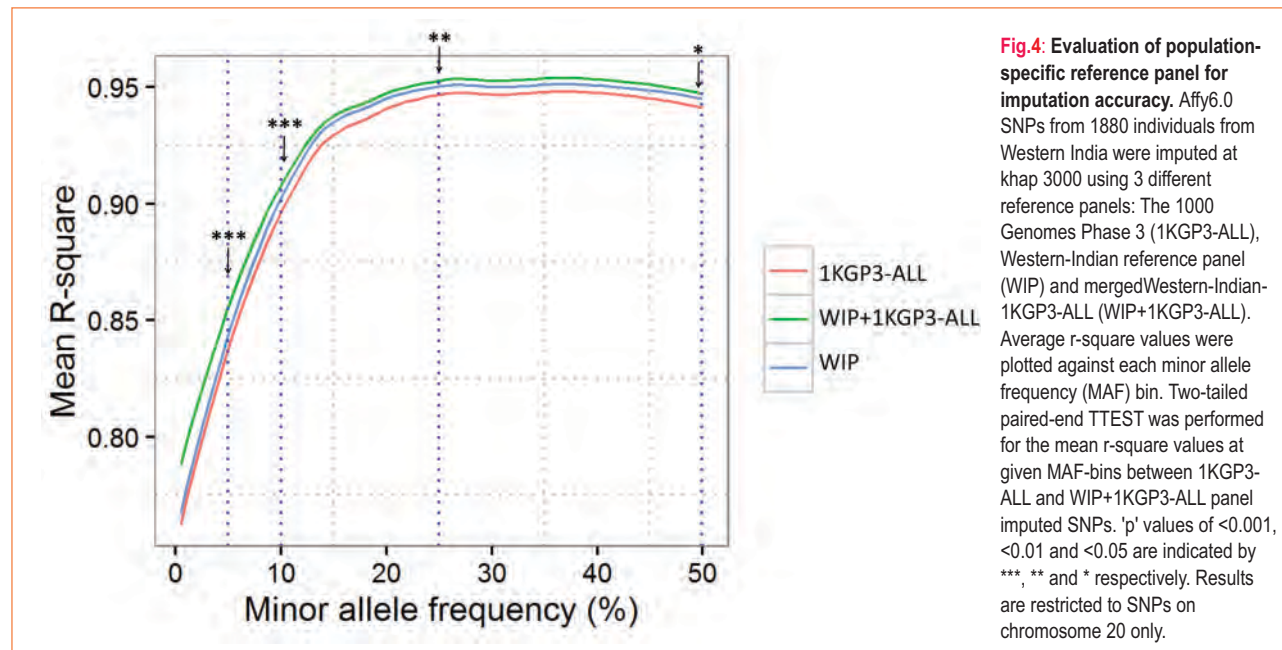
Inclusion of population-specific reference panel from India to the 1000 Genomes Phase 3 panel improves imputation accuracy

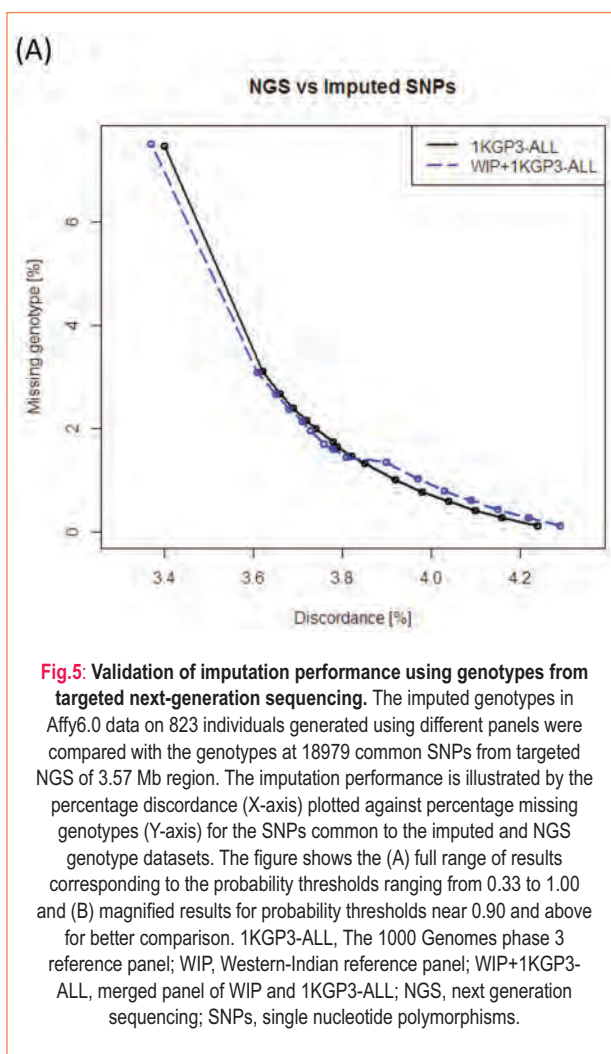
Genome-Wide Association Studies (GWASs) have identified many disease risk loci for various complex diseases and traits. Several population specific risk loci have also been reported, which brings GWASs to a phase where population-specific loci expand the understanding of disease mechanisms and pathways. The content of the variants may not overlap across different GWAS arrays which make it difficult to compare or meta-analyze two or more GWASs. Imputation is a cost-effective computational strategy based on the pattern of LD structure and sharing of haplotype stretches among individuals. It allows analyzing a larger number of variants without genotyping them directly by using the GWAS data alongside a comprehensive dataset called as reference panel and thus increases the power of GWASs, meta-analyses and fine mapping studies. Researchers have been using The 1000 Genomes phase 1 reference panel for imputation with reasonable accuracy. Recent independent studies using Japanese population reference panel (1KJPN) from 1070 Japanese individuals and Genome of Netherlands (GoNL) panel from 769 Dutch individuals have been shown to add to the imputation accuracy. It is thus important to have appropriate reference panel for accurate imputation since features such as haplotype structure, presence of population-specific variants and altered frequency of variants influence the imputation quality and genomic coverage of the imputed variants. Owing to second highest genetic diversity in India after African populations, including

more Indian samples from different ethnic backgrounds and sub-populations can enhance the imputation performance.

We generated Western-Indian Reference Panel (WIP), a population-specific panel by combining the Affy6.0 data with Illumina HumanCoreExome data on 407 individuals from the PMNS cohort. The combined dataset includes 931,371 high-quality autosomal single nucleotide polymorphisms (SNPs). The SNPs from Affy6.0 chip on another 1880 individuals on chromosome 20 were imputed using IMPUTE2. The r-square is the metric calculated as the squared correlation between input and masked/imputed genotypes at a SNP which is used to measure imputation accuracy. It varies between 0-1, where values near 1 indicate that a SNP has been imputed with high certainty. Comparison of the r-square values averaged for each minor allele frequency (MAF) bin for SNPs common between the imputed datasets (18266 SNPs) shows that the WIP confers marginal enhancement in imputation performance than the 1000 Genomes phase 3 panel (KGP3-ALL) (Figure 4). The accuracy was further enhanced significantly across the MAF spectrum when the combined panel, WIP+1KGP3-ALL was used ($p < 0.05$ for $>93\%$ MAF bins).

Imputation performance was assessed using GWAS array SNPs but a comparison of the imputed SNPs in certain genomic regions with much denser experimental genotype data available from next generation sequencing (NGS) is desirable. Hence, we compared concordance between imputed genotypes and the direct genotypes obtained through targeted NGS for a 3.57 Mb region spanning





chromosomes 3, 5 and 10 on 823 subjects. For a given missingness threshold, percentage discordance was lesser for WIP+1KGP3-ALL as compared to the 1KGP3-ALL panel indicating that concordance between imputed and true genotypes is higher with the WIP+1KGP3-ALL panel (Figure 5). This study stresses on the existence of population substructure among the Indian populations and on the need for a more comprehensive reference panel from Indian populations with denser genotype information based on whole genome sequence data. Such a panel could then be applied to populations of South-Asian diaspora which can enhance the imputation accuracy.

A functional enhancer variant within *SPINK1* c.101A>G (p.Asn34Ser)-containing haplotype influences the risk of chronic pancreatitis

Chronic pancreatitis (CP) is an inflammatory disease of pancreas that results in irreversible structural and

functional damage to pancreatic parenchyma. Mutations in several genes including those predominantly expressed in pancreas - cationic trypsinogen (*PRSS1*), anionic trypsinogen (*PRSS2*), Serine protease inhibitor Kazal type I (*SPINK1*) and Chymotrypsin C (CTRC) have been reported to be associated with chronic pancreatitis worldwide. We have earlier demonstrated absence of mutations in cationic and anionic trypsinogen genes in Indian CP patients using tropical calcific pancreatitis (TCP) as model, and hence *SPINK1* mutations, especially the *SPINK1* c.101A>G (rs17107315: A>G; p.Asn34Ser) variant is likely to play a very important role in the genetic susceptibility of TCP. Although, the *SPINK1* rs17107315 variant-associated haplotype has emerged as the strongest risk predictor, despite extensive studies, functional variant within this haplotype has remained elusive. Therefore, we explored the possibility that the causal variant could be residing within an uncharacterized flanking region of the *SPINK1* gene and may have a regulatory potential. On the basis of results obtained from HaploReg v4.1 with LD threshold of $r^2 \geq 0.40$ using the 1000 Genomes Project Phase 1 data, and bioinformatic prediction for transcription factor binding sites as well as visual inspection, *SPINK1* c.-4141C>A (rs142703147:C>A) was identified as one of the potential candidate variant. We genotyped the above-mentioned variant in 347 Indian CP patients and 264 well-characterized controls. We observed significantly increased risk (OR=14.82; $P=2.04 \times 10^{-16}$) of CP in carriers of 'A' allele at rs142703147 variant. Further, visual inspection of the local DNA sequence spanning the rs142703147 variant showed disruption of putative transcription factor binding site for the pancreatic-specific trimeric complex, PTF1L thus suggesting a likely functional role for this variant. However, unlike in the Europeans where it is in perfect linkage disequilibrium (LD; $r^2=1$) with rs17107315, the two variants are in moderate LD ($r^2=0.59$) in Indians. Consequently, although the variant rs142703147:C>A appears to be of functional significance, observations from population genetic studies clearly suggest that it is essentially one component of the chronic pancreatitis-predisposing functional elements contained within the risk haplotype of interest. Thus, we are still far from deciphering the pathogenic mechanisms underlying *SPINK1* gene, the strongest heritable risk factor for CP.

Amitabha Chattopadhyay

Membrane and Receptor Biology



From top (left to right): Md. Jafurulla, Amitabha Chattopadhyay, Sandeep Shrivastava, Suman Bandari, G. Aditya Kumar, Bhagyashree D. Rao, Sreetama Pal, Parijat Sarkar, Thirupathi Reddy, Shroddha Bose, M. Ranga Rao, Nikita P. Chutake, K. Venkatlaxshmi

RESEARCH INTERESTS:

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

The overall research interest of our group is centered on membrane and receptor biology using a variety of biophysical, biochemical and cell biological approaches. Fluorescence-based spectroscopic and microscopic approaches are extensively used for this purpose. A major area of research is the interaction of G protein-coupled receptors (GPCRs) with membrane lipids and its implications in health and disease. An interesting application of this work is in the role of membrane lipids and cytoskeleton in the entry of intracellular pathogens.

Selected recent publications

1. Chaudhuri A., Prasanna X., Agiru P., Chakraborty H., Rydström A., Ho JCS., Svanborg C., Sengupta D., and Chattopadhyay A. (2016) "Protein-dependent Membrane Interaction of A Partially Disordered Protein Complex with Oleic Acid: Implications for Cancer Lipidomics" *Sci. Rep.* 6: 35015.
2. Kumar GA., Roy S., Jafurulla M., Mandal C., and Chattopadhyay A. (2016) "Statin-induced Chronic Cholesterol Depletion Inhibits *Leishmaniadonovani* Infection: Relevance of Optimum Host Membrane Cholesterol" *Biochim. Biophys. Acta(Biomembranes)* 1858: 2088-2096.
3. Prasanna X., Sengupta D., and Chattopadhyay A. (2016) "Cholesterol-dependent Conformational Plasticity in GPCR Dimers" *Sci. Rep.* 6: 31858.
4. Pydi SP., Jafurulla M., Wai L., Bhullar RP., Chelikani P., and Chattopadhyay A. (2016) "Cholesterol Modulates Bitter Taste Receptor Function" *Biochim. Biophys. Acta* 1858: 2081-2087.
5. Viswanathan G., Jafurulla M., Kumar GA., Raghunand TR., and Chattopadhyay A. (2015) "Dissecting the Membrane Cholesterol Requirement for Mycobacterial Entry into Host Cells" *Chem. Phys. Lipids* 189: 19-27.

Organization and Dynamics of Membranes and Proteins utilizing the Wavelength-Selective Fluorescence Approach

Our group pioneered the application of wavelength-selective fluorescence as a novel approach to monitor organization and dynamics of probes and proteins in membranes and membrane-mimetics such as micelles and reverse micelles. Wavelength-selective fluorescence relies on the slow rates of solvent relaxation around an excited state fluorophore, which is a function of the motional restriction imposed on the solvent molecules in the immediate vicinity of the fluorophore. Utilizing this approach, it becomes possible to probe the mobility parameters of the environment itself (which is represented by the relaxing solvent molecules) using the fluorophore merely as the reporter group. Further, since the ubiquitous solvent for biological systems is water, the information obtained in such cases will come from the otherwise 'optically silent' water molecules. This makes the use of wavelength-selective fluorescence approach significant in biology since hydration plays a crucial modulatory role in a large number of vital cellular events. Important applications of this approach include monitoring the environment of the functionally relevant tryptophans in the prototypical ion channel gramicidin and in the lytic peptide melittin from bee venom. Interesting applications include monitoring defined depths in the membrane utilizing depth-dependent solvent relaxation as a dipstick and lipid-protein interactions in membranes.

In addition, our group applied the wavelength-selective fluorescence approach to monitor organization and dynamics of functionally important tryptophan residues in tubulin, erythrospectrin and -lactalbumin. In a recent work, the wavelength-selective fluorescence characteristics of the green fluorescent protein (GFP) was monitored. Results show that the slow dipolar relaxation of GFP is due to the rigid protein matrix of GFP around its fluorophore, independent of the viscosity of the surrounding medium. In a recent work, the rotational dynamics of Golgi membranes was measured using the principle of wavelength-selective fluorescence.

Interaction of Membrane Cholesterol with the Serotonin_{1A} Receptor

Membrane proteins mediate a wide range of essential cellular processes such as signaling across the

membrane, cell-cell recognition, and membrane transport. About 30% of all open reading frames (ORFs) are predicted to encode membrane proteins and almost 50% of all proteins encoded by eukaryotic genomes are membrane proteins. Importantly, membrane proteins represent prime candidates for the generation of novel drugs in all clinical areas. Since a significant portion of integral membrane proteins remains in contact with the membrane, the structure and function of membrane proteins depend on their interactions with the surrounding lipids. The serotonin_{1A} receptor, an important neurotransmitter receptor, is a member of a superfamily of seven transmembrane domain receptors that couple to GTP-binding regulatory proteins (G-proteins). Although G-protein-coupled receptors (GPCRs) represent ~50% of current drug targets, only a small fraction of all GPCRs are presently targeted by drugs. Serotonergic signaling plays a key role in the generation and modulation of various cognitive, behavioral and developmental functions. Disruptions in serotonergic systems have been implicated in the etiology of mental disorders such as schizophrenia, migraine, infantile autism, eating disorders, and obsessive compulsive disorder. Although none of the serotonin receptors have been purified to homogeneity from native sources yet, we have been able to partially purify and solubilize functional serotonin_{1A} receptors.

Seminal work from our laboratory has comprehensively demonstrated the requirement of membrane cholesterol in the function of the serotonin_{1A} receptor. In addition, a cellular model for the Smith-Lemli-Opitz Syndrome (SLOS, a disease associated with defective cholesterol biosynthesis), was generated to further address this issue. SLOS is an autosomal recessive disorder characterized clinically by mental retardation, physical deformities, failure to thrive and multiple congenital anomalies. Ligand binding activity, G-protein coupling and downstream signaling of serotonin_{1A} receptors are found to be impaired in the cellular model of SLOS. These results could be potentially useful in understanding the molecular basis that underlie the pathophysiology

of SLOS and could provide novel insight in formulating future treatment for the disease. In addition, it was shown that 7-dehydrocholesterol (7-DHC), the immediate biosynthetic precursor of cholesterol, could not support the function of the serotonin_{1A} receptor. These results comprehensively demonstrate the specific requirement of membrane cholesterol for the function of serotonin_{1A} receptor, although global membrane effects cannot be completely ruled out. In an interesting and developing aspect of this work, our group has addressed the issue of stringency criterion of the molecular structure of cholesterol in supporting the function of the serotonin_{1A} receptor. A prominent site among these is the cholesterol recognition/interaction amino acid consensus (CRAC) motif, recently identified by this group in GPCRs. Recent work from our group has shown that cholesterol binding sites in GPCRs are highly dynamic, and have a microsecond time scale of exchange with bulk membrane lipids. In addition, GPCR dimers exhibit cholesterol-dependent conformational plasticity. These results have potential implications for the design of therapeutic strategies for tissue-specific and age-dependent interventions.

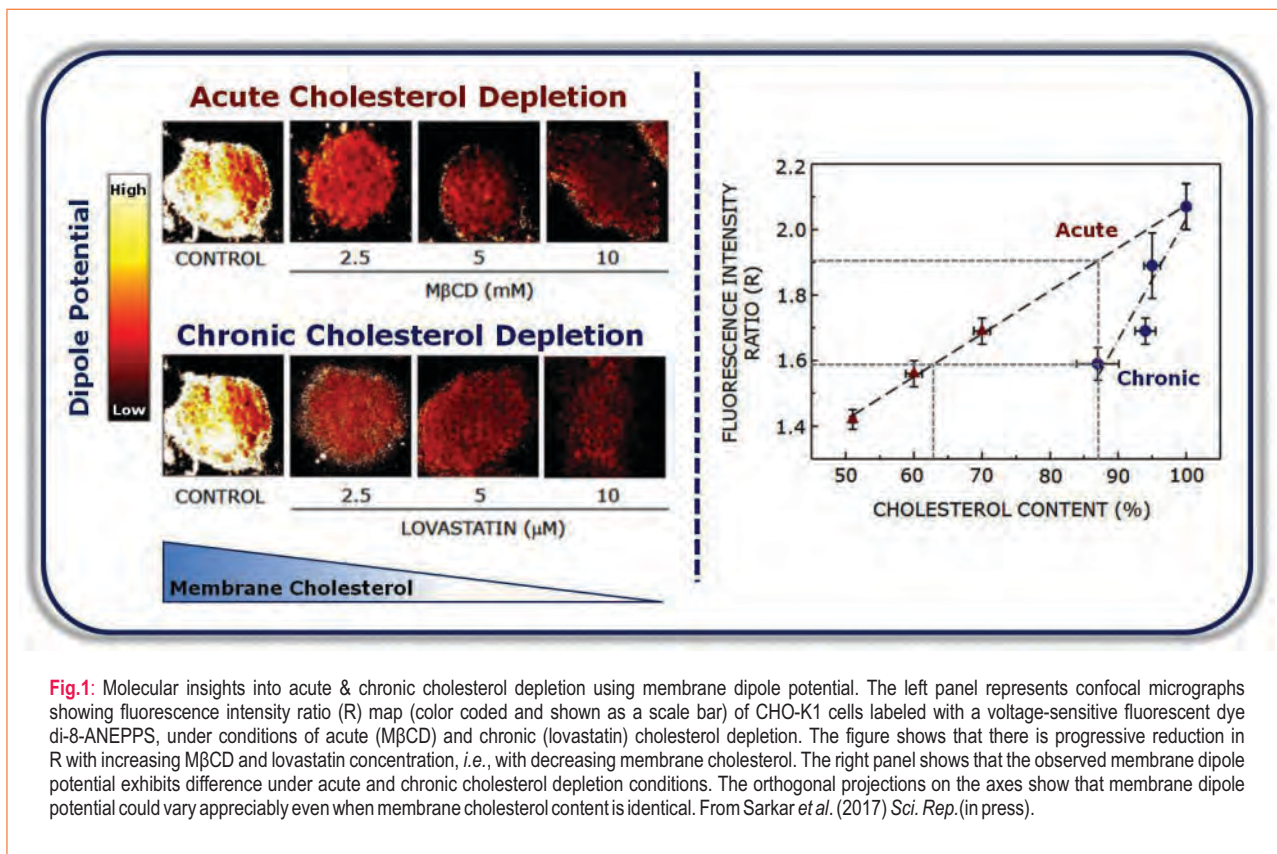
Exploring the Effect of Local Anesthetics on Serotonin_{1A} Receptor Function

Local anesthetics belong to a group of amphiphilic compounds which suppress the feeling of pain, when applied in a particular part of the body by preventing the transmission of nerve impulse, thereby reducing the pain in that area. In spite of a large amount of research, the molecular mechanism behind the action of local anesthetics is not well understood. Phenylethanol (PEtOH) is a constituent of essential oils having a pleasant odor and can act as a local anesthetic. In recent work, we have studied the effect of PEtOH on the function of hippocampal serotonin_{1A} receptor, a representative neurotransmitter receptor belonging to the G protein-coupled receptor (GPCR) family. The results obtained show that PEtOH causes a reduction in ligand binding to the serotonin_{1A} receptor due to lowering of agonist binding

affinity, and a decrease in the extent of G-protein coupling. Environment-sensitive fluorescent probe DPH showed a reduction in membrane order with increasing PEtOH concentration, as apparent from the decrease in rotational correlation time of the probe. An overall analysis of results obtained reveal that the action of local anesthetics could be ascribed to the combined effects of specific interaction of the receptor with anesthetics and change in membrane physical properties (such as membrane order). These results are relevant in the context of anesthetic action and could be beneficial to accomplish a better understanding of the possible role of anesthetics in the function of neuronal receptors.

Role of Sphingomyelinase of *Leishmania donovani* in its Entry into Host Cells

Leishmania donovani is an obligate intracellular parasite that causes visceral leishmaniasis in humans, a major cause of mortality and morbidity worldwide, particularly among economically weaker sections in tropical and subtropical regions. Visceral leishmaniasis is a potent disease which is fatal if untreated. The molecular mechanism involved in the interaction of *Leishmania* with the plasma membrane of host cells during its internalization is poorly characterized. Previous work from our group has demonstrated the role of membrane cholesterol and actin cytoskeleton of macrophages in the entry and survival of the *Leishmania* in host cells. In a recent work, we (in collaboration with Dr. Chitra Mandal's group at CSIR-IICB) identified, for the first time, an active neutral sphingomyelinase (NSMase) enzyme in *Leishmania donovani*. Since *Leishmania* does not have sphingomyelin, the parasite could utilize its NSMase to target macrophage sphingomyelin for its entry into host cells. In line with this, our results show that an active NSMase is essential for the entry of *L. donovani* into host macrophages. These results help understand the role of NSMase enzyme of the parasite in infecting host macrophages and assume relevance in the context of developing future therapeutic strategies to tackle leishmaniasis.



Differential Membrane Dipolar Orientation Induced by Acute and Chronic Cholesterol Depletion

Cholesterol plays a crucial role in cell membrane organization, dynamics and function. Depletion of cholesterol represents a popular approach to explore cholesterol-sensitivity of membrane proteins. An emerging body of literature shows that the consequence of membrane cholesterol depletion often depends on the actual process of depletion, (acute or chronic), although the molecular mechanism underlying the difference is not clear. With the overall goal of addressing molecular differences underlying these processes, recent work

from our group has demonstrated differential membrane dipole potential under conditions of acute and chronic cholesterol depletion in CHO-K1 cells, using a voltage-sensitive fluorescent dye in dual wavelength ratiometric mode. These results show that the observed membrane dipole potential exhibits difference under acute and chronic cholesterol depletion conditions, even when cholesterol content is identical. These results provide molecular insight highlighting differences between these processes, and help in comprehensive understanding of processes in which membrane cholesterol gets modulated.

Mandar V Deshmukh

Post-transcriptional Gene Silencing Mediated by RNA-Binding Proteins



From left to right: K. Joy, Akanksha Bhisht, Sneha Paturi, Mandar V Deshmukh, Ramdas Aute, Upasana Rai

RESEARCH INTERESTS:

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

How do RNA-binding proteins effect 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

1. Sai Chaitanya C, Ramdas A, Upasana R and Mandar V Deshmukh (2017) DRB4 dsRBD1 drives dsRNA recognition in *Arabidopsis thaliana* siRNA pathway *Nucleic Acids Research*, 45(14), 2017.
2. Sai Chaitanya C and Mandar V Deshmukh (2016) Recent Excitements in protein NMR: Large proteins and biologically relevant dynamics. *Journal of Biosciences*, 41(4); 787-803.
3. Sai Chaitanya C and Mandar V Deshmukh (2015) Chemical shift assignments of DRB4 (1-153), a dsRNA binding protein in *A. thaliana* RNAi pathway. *Biomolecular NMR Assignment* 9(2), 253-6, 2015.
4. Rakhi Sharma, B Sahu, Malay K Ray and Mandar V Deshmukh (2015) Backbone and stereospecific ¹³C methyl Ile (1), Leu and Val sidechain chemical shift assignments of Crc. *Biomolecular NMR Assignments*, 9(1), 75-9, 2015.
5. Sai Chaitanya C and Mandar V Deshmukh (2014) Structure of RDE-4 dsRBDs and mutational studies provide insights in the dsRNA recognition in *C. elegans* RNAi *Biochemical Journal* 458(1), 119-130.

(Highlighted under "Spotlight" section of BJ Biomolecules in the same issue)

Our lab explores the evolutionary divergence in the process of RNAi initiation in various organisms. It is known that Watson-Crick base pairing in dsRNA predominantly assumes form-A of double helical nucleic acid structure. This 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 $\alpha\beta\beta\beta\alpha$ structure where two α helices organize to form an $\alpha1\alpha2$ interface that packs against antiparallel beta sheet formed by $\beta1\beta2\beta3$. dsRBDs recognize dsRNA through the helical face with three steric contacts in which residues Q/H and E in $\alpha1$ contact minor groove, KKxxK belonging to the N-terminus of $\alpha2$ bind to the major groove, and the H of the $\beta1\beta2$ loop contacts subsequent minor groove of dsRNA.

The ability to recognise exclusive structural features on dsRNA in an RNA sequence independent manner makes dsRBDs as the domain of choice for several important regulatory enzymes, e.g., Dicers. Interestingly, despite containing multiple dsRNA binding domains at their C-terminal region, across all organisms, Dicers heavily depend on an additional class of auxiliary proteins, dsRBPs (dsRNA Binding Domain Containing Proteins), so heavily that the activity of Dicer is driven by these dsRBPs. 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 *Arabidopsis* interact with five dsRBPs (HYL1, DRB2 to DRB5). Human Dicer requires dsRBPs, 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. Despite similarities, dsRBPs are seen to exhibit a wide spectrum of affinities with dsRNA. The differential affinity allows some dsRBPs to selectively bind to a longer dsRNA (initiation of RNAi through Dicer, an upstream process) while others bind to a smaller dsRNA (transportation of siRNA to Ago, a downstream process). 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. Our lab has been exploring the molecular basis of

dsRNA binding proteins in the RNAi pathway of various organisms.

Previously, we have solved the solution structure of RDE-4, which initiates RNAi in *C. elegans*, and showed that both dsRBDs possess additional structural elements that are responsible for the dsRNA binding ability. The solution structure of dsRBD2 suggests that the linker embraces the dsRBD fold while allowing its access to Dcr-1. The structural arrangement further confers accessibility to the canonical dsRNA binding surface for the simultaneous dsRNA interaction. Interestingly, 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. Thus, our study rationalized the higher affinity demonstrated by dsRBD2 and sheds light on Dicer recognition process in the RNAi initiation.

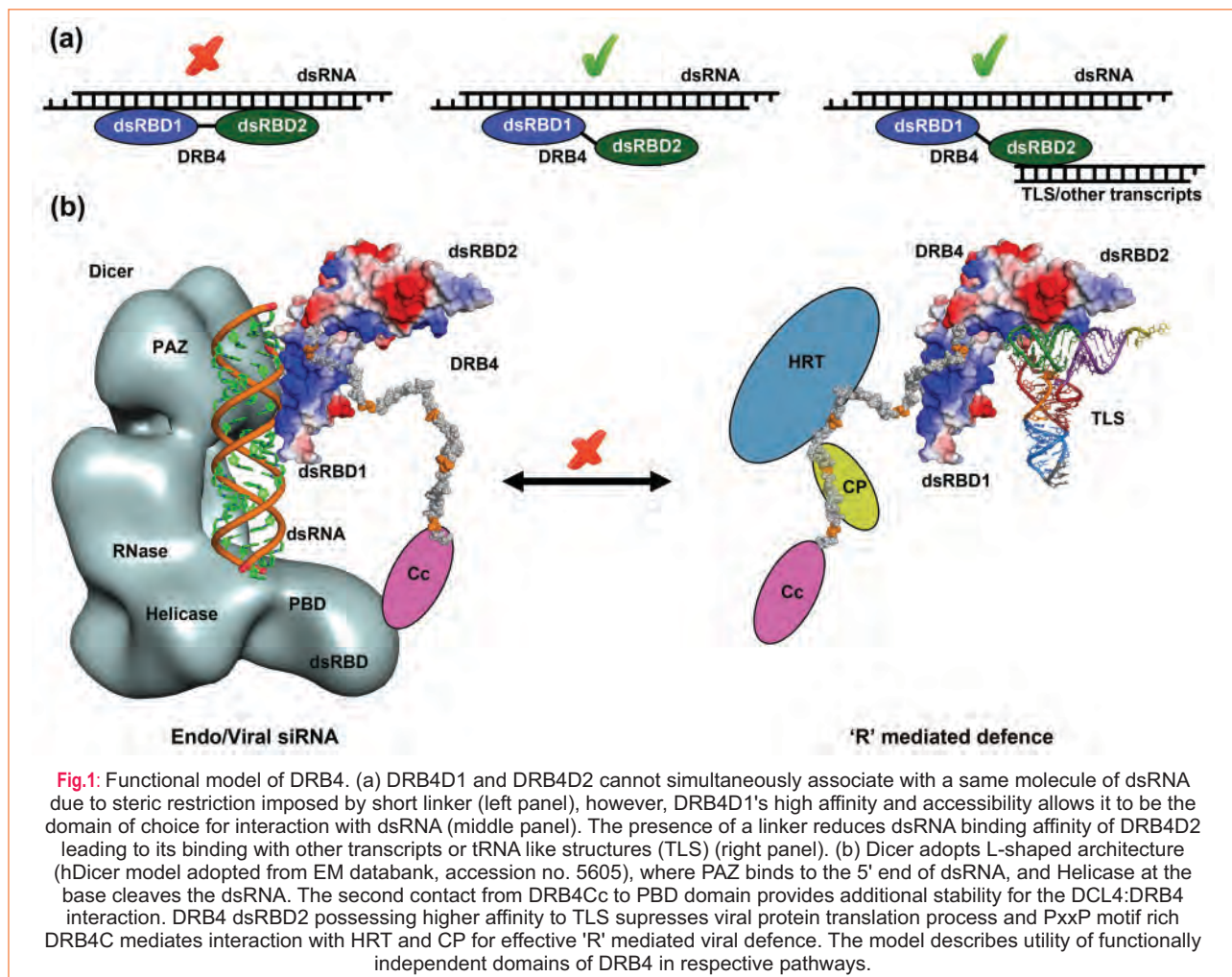
Plants depend on DRB4 in achieving efficient tasi/siRNA dependent viral defense response by two independent and spatially separated pathways (i.e., tasi/siRNA mediated gene silencing in the nucleus) and 'R' mediated antiviral response (in the cytoplasm). During the previous year, we have solved the solution structure of tandem dsRBDs of DRB4 (DRB4D1D2), which consists of two dsRBDs (DRB4D1 and DRB4D2) connected by a linker region of nine amino acids. While the solution structure reveals that both dsRBDs fold into a canonical $\alpha\beta\beta\beta\alpha$ structure, DRB4D1 exhibits higher affinity with two binding modes (0.3 and 3 μM) for 20 bp dsRNA over dsRBD2 ($\sim 1 \mu\text{M}$). The differential affinity exhibited by structurally similar DRB4 dsRBDs was quite unexpected and hence we probed if DRB4D1 possesses any advantages in terms of slow time scale dynamics. The ^{15}N CPMG data suggests that despite having similar structural motifs, the differential affinity exhibited by both dsRBDs stem from subtle structural differences coupled with favourable conformation sampling at the RNA binding regions. The favorable structural arrangement and presence of dynamics at ms- μs time scale makes dsRBD1 as the domain of choice for the dsRNA interaction in DRB4. The low amplitude slow motions provide the necessary flexibility to the $\alpha1$ helix in dsRBD1 so that it can be accommodated onto the minor groove of dsRNA. On the contrary, despite possessing a canonical dsRBD fold, dsRBD2 lacks the ideal sidechains orientation in the key RNA binding residues as well as the domain exhibits no significant motions.

Interestingly, the weaker affinity of DRB4D2 befalls

weaker in DRB4D1D2 and the electrophoretic mobility shift assay indicated that about only 30% of dsRBD2 is accessible to dsRNA due to the presence of dsRBD1. Further validation of key RNA binding residues by the gel shift assays using several DRB4D1D2 single and double active site mutants confirmed that dsRBD1 contributed to overall 70% activity and dsRBD2 contributes to ~ 30% activity in dsRNA:dsRBP complex. Interdomain PRE emanating from multiple sites of both domains suggested that the reduced flexibility of the linker might induce a preferred interdomain orientation. DRB4D1D2 structure suggests that conformations adopted by the linker possibly hinder the key dsRNA recognition amino acids in $\alpha 1$ of dsRBD2. In such scenario, dsRBD2:dsRNA complex formation is driven by contacts made only by the N-terminus of $\alpha 2$ and $\beta 1$ - $\beta 2$ loop and would result in reduced binding. Further, the steric hindrance induced by the linker additionally reduces dsRNA recognition ability of DRB4D2. Therefore, we postulate that dsRBD2 may be recruited to recognize other RNA transcripts which does not require tripartite contact. The studies on R

protein mediated viral defense show that the viral RNA translational enhancer RNA adapt a tRNA-like structure (TLS) and is exclusively recognized by DRB4D2, but not DRB4D1, to repress viral RNA translation. Therefore, we propose that limited access of dsRBD2 to dsRNA may be an advantage for its strong association to viral tRNA-like structures containing stem loops and mismatches.

In case of DRB4, the native trigger dsRNA for DRB4:DCL4 complex is always > 60 bp dsRNA with perfect palindromic sequence. Our results suggest that DRB4 dsRBDs use a complex mechanism to bind to longer dsRNA than previously studied dsRBPs such as TRBP and DRB1. While a single dsRNA can be associated with four molecules of individual and tandem dsRBDs, which is in agreement with TRBP, the significant resonance broadening during dsRNA binding observed for DRB4D1 and DRB4D1D2 is puzzling. The resonance broadening coupled with multiple binding events allow DRB4 dsRBD1 to slide over long dsRNA. As stated earlier, dsRBD1 is the principle dsRNA recognizer and the steric hindrance induced by the linker does not allow dsRBD2



to bind to the same molecule that is bound by dsRBD1. In this scenario, the complex formed by DRB4D1D2 with longer dsRNA will be highly heterogeneous and dynamic. The resonance broadening in NMR based titrations with 20 bp dsRNA is a result of multiple simultaneous events, i.e., binding of multiple dsRBD1 (up to four) with a single molecule of dsRNA, the binding kinetics, the sliding of dsRBD1 over the length of dsRNA and transient interactions of free dsRBD2 with another molecule of dsRNA. The multiple binding events and the sliding are also reflected in the broadening of imino protons of dsRNA and α -helical binding face in dsRBD1, whereas remaining residues of dsRBD1, linker and dsRBD2 experience broadening due to the formation of large macromolecular assembly. Given the complex nature of the binding, it is difficult to

draw more definitive conclusion on the binding event of DRB4D1D2 with longer dsRNA. Our study elucidates 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.

In another project, the lab is exploring structural details of DRB2 which synchronously acts with DRB1 in *Arabidopsis* miRNA pathway. So far, we have derived the solution structure of the 1stdsRBD and 2nddsRBD appears as a higher order oligomer in NMR as well as in gel filtration studies. The project continues in exploring functional significance of the abnormal structural features in DRB2 and its role in miRNA biogenesis in plants.

Jyotsna Dhawan

Molecular Programs of Quiescence in Adult Stem Cells and Regeneration



From left to right: Jyotsna Dhawan, Ch. Sudhakar, Debarya Saha, Sujoy Deb, Ananga Ghosh, Ajoy Aloysius, Amena Saleh, Gunasekaran Subramaniam, Swetha Sundar, Prabhavathy Devan, Nandini Rangaraj, A.S. Priti, Swetha Gopal, Gunjan Purohit (not present: Lamuk Zhaveri)

RESEARCH INTERESTS:

- Control of cellular quiescence and its relationship to stem cell function.
- Adult stem cells and tissue regeneration.
- Secreted and mechanical signals in control of cell fate
- Exosomes in tissue repair

Our group is 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 to investigate the links between two key features of quiescence - repression of differentiation and the potential to return to active division.

Selected recent publications

(joint affiliation of JD with CCMB and inStem)

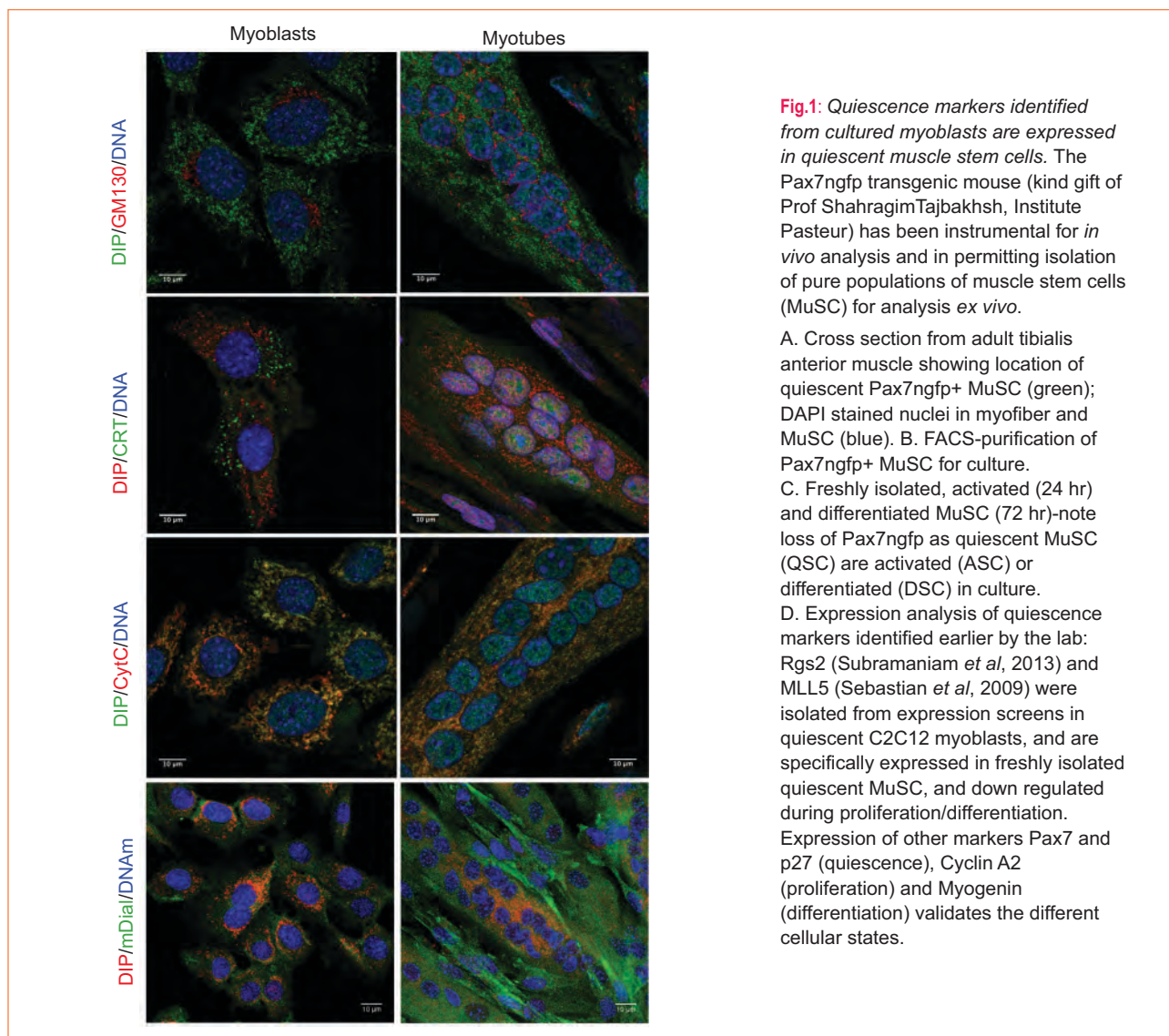
1. Sowpati DT, Srivastava S, Dhawan J and Mishra RK (2017) C-State: An interactive web app for simultaneous multi-gene visualization and comparative epigenetic pattern search, *BMC Bioinformatics* (in press).
2. Srivastava S, Gala H, Mishra RK and Dhawan J (2016) Distinguishing states of arrest: Genome-wide descriptions of cellular quiescence using ChIP-seq and RNA-seq analysis *Methods in Molecular Biology*, Daniel Lacorazza (Ed) (in press) (Invited article).
3. Vyas, N and Dhawan J (2016) Exosomes: mobile platforms for targeted and synergistic signaling across cell boundaries. *Cell. Mol. Life Sci.* DOI 10.1007/s00018-016-2413-9.
4. Sellathurai J, Nielsen J, Dhawan J, Nielsen MFB, and Schroder HD(2016) Low oxygen tension enhances expression of myogenic genes when human myoblasts are activated from G₀ arrest. *PLoS ONE* 11(7):e0158860.

Our group is interested in the mechanisms that regulate cellular quiescence in 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 also repair 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.

We use genome-wide strategies coupled with functional analysis to investigate the links between

two key features of quiescence -repression of differentiation and the potential to return to active division. Using myogenic cell lines, muscle stem cells (Fig.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 altered by cell cycle re-entry.

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.



Chromatin and transcriptional mechanisms in G₀

Earlier we reported that an epigenetic regulator PRDM2 controls the decision between two key aspects of quiescence- repressed differentiation and poising of the cell cycle, and defined a new mechanism by which PRDM2 governs this balance, by targeting the bivalent marking of a PRE-like element in the Cyclin A regulatory region (Cheedipudi, Puri et al, Nucleic Acids Res, 2015). Over the past year, we have extended these studies in three ways (i) immuno-precipitation of tagged PRDM2 isoforms to identify interacting partners using mass spectrometric analysis (ii) site-directed mutagenesis to define the PRE-like regulatory element where PRDM2 is bound (iii) defining the signaling pathways directly downstream of PRDM2.

We are deciphering the rules that may govern the distinct chromatin landscape in quiescent stem cells using a population of cardiac MSC, in collaboration with Richard Harvey at VCCRI, Sydney and Rakesh Mishra at CCMB. The results reinforce our view that global patterns of histone modifications induce a poised state in G₀. Using this data set, in collaboration with Rakesh Mishra's group we have devised a means of visualizing the distribution of multiple epigenetic marks in multiple cellular states and thereby discerning patterns that allow the generation and testing of hypotheses about global regulation at the level of chromatin (Sowpati et al, 2017).

We have found that promoter-proximal pausing of RNA pol II is involved in the maintenance and exit from the quiescent self-renewing state. By combining RNAseq and ChIP-seq of RNA polymerase occupancy in different cellular states, we uncovered networks that point to quiescence-specific priming of the transcriptional program. Knockdown analysis of G₀-stalled genes shows that stalling contributes to self-renewal (Gala et al, in revision). Over the past year we have extended our analysis to up-stream transcriptional regulators and find that control of polymerase stalling determines the timing of entry into the cell cycle.

RNA biology in quiescence

Earlier, we reported cell state-specific dynamics of self-assembling mRNP granules known as P-bodies. We also discussed evidence that in quiescence transcript stabilization in mRNPs dominates over

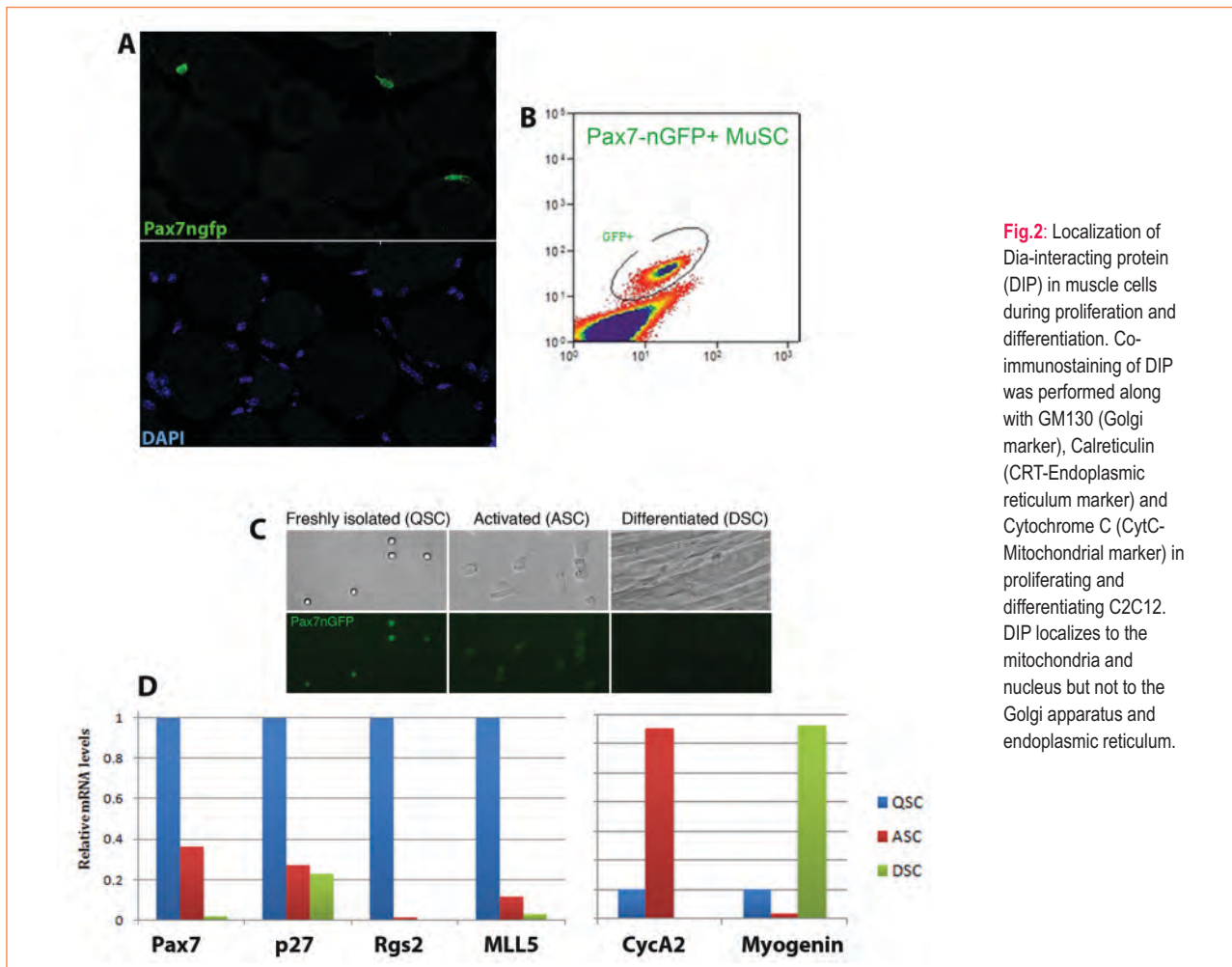
transcript turnover. In culture, knock-down of different P-body components that are specifically enriched in muscle stem cells *in vivo* differentially affects proliferation and quiescence. Studies with knockout mice indicated a role in muscle homeostasis. Over the past year we have uncovered a cross-regulation between different mRNP granule components that implies a stage-specific mechanism to monitor their expression, assembly and function.

Secreted and mechanical signals in control of cell fate

Secreted ligands such as Wnt and bFGF are known to be critical for muscle cell proliferation. Our earlier studies established that threshold levels of Wnt- β -catenin-TCF signaling are important for the quiescence program and appear to be associated with a very different spectrum of genes in quiescent myoblasts when compared to either cycling or differentiated muscle cells. Consistent with the surprising finding that β -catenin is dispensable for this pathway and instead cross talk with other signaling systems is required in G₀ (Aloysius *et al*, in revision). Our studies over the past year have revealed that this transcriptional cross-talk is likely to occur in muscle stem cells during post-natal development.

Mechanical signals from the substratum have a profound impact on cytoskeletal and nuclear architecture and consequently on cell fate. Earlier we had reported the generation of culture systems that permit human mesenchymal stem cells to be toggled between quiescence and activation *in vitro*, by limiting cell attachment or by culture on soft substrates (in collaboration with Moustapha Kassem at Odense). We have uncovered quiescence-induced transcriptional programs associated with a clinically relevant phenotype (Rumman *et al*, in preparation). Over the past year, we have also found that inducing quiescence in culture enhances the function of transplanted hMSCs *in vivo*.

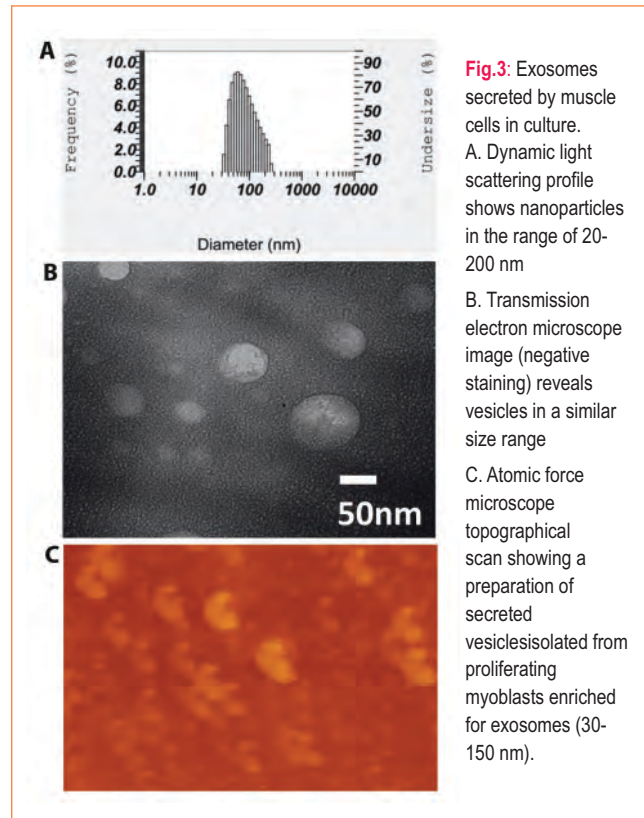
Cytoskeletal signaling is integrated with growth factor signaling via the activity of the RhoA GTPase. Earlier we had identified mDiaphanous (mDia) as the key effector of RhoA in mediating signaling via regulation of transcription factors that are responsive to the polymerization state of the actin cytoskeleton (Gopinath *et al*, *J. Cell Sci*, 2007). More recently, using yeast 2-hybrid analysis we have isolated a novel interacting protein of mDia, confirmed their interaction by immuno-precipitation and mass



spectrometry, and mapped the interacting domains. In particular, this novel protein appears to regulate a stage-specific signaling complex to controls differentiation. Fig. 2 shows the localization of the mDia-interacting protein at different stages of myogenesis in culture. Our current studies are aimed at understanding how the interaction of mDia with DIP regulates muscle differentiation.

Secreted exosomes and tissue regeneration

Earlier work initiated by N. Vyas while the lab was at in Stem revealed that extracellular vesicles (“exosomes”) are secreted in at least two distinct forms with unique protein and miRNA cargoes and distinct signaling capabilities (Vyas et al, *Sci Rep*, 2014). As part of an Indo-Danish collaborative grant with 4 Danish and 5 Indian labs, we are now extending our studies towards understanding whether stem cells improve tissue repair by secreting exosomes that stimulate more efficient regeneration. Fig.3 shows preliminary characterization of exosomes from cultured muscle cells. Our studies over the next year will focus on the exosomal cargo of proteins and miRNAs.



G Umapathy

Studies on Species Extinction and Conservation Physiology



From left to right: Neema, Abirami, M.S Ram, Mahender Reddy, Mihir Trivedi, G. Umapathy, Vinod Kumar, Deepa Purohit, Pravalika, Kirti Prasad

RESEARCH INTERESTS:

- Understanding the extinction process of species.
- Impact of habitat fragmentation in Western Ghats.
- Conservation breeding.
- Conservation physiology.
- Understanding anthropogenic disturbance to wildlife health and reproduction.

Our group aims to understand factors that influence the extinction of species in fragmented landscape, and to assess reproductive and stress status in wild animals.

Selected recent publications

1. Chakraborty D, Tiwari S, Reddy DM, Umapathy G (2016) Prevalence of gastrointestinal parasites in civets of fragmented rainforest patches in Anamalai hills, Western Ghats, India. *Journal of Parasitology* 102: 463-467.
2. Ram MS, Kittur SM, Biswas J, Nag S, Shil J, Umapathy G (2016). Genetic Diversity and Structure among Isolated Populations of the Endangered Gees Golden Langur in Assam, India. *PLoS ONE* 11(8): e0161866. doi:10.1371/journal.pone.0161866.
3. Budithia NMR, Kumar V, Yalla SKA, Rai U and 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.
4. Kumar, V, Reddy, V, KokkiligaddaA, Shivaji, S and 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.
5. Laurance WF, Umapathy G, et al. (2012). Averting biodiversity collapse in tropical forest protected areas. *Nature* 489: 290-294.

Genetic diversity and structure among isolated populations of the endangered Gee's golden langur in Assam, India

Gee's golden langur (*Trachypithecus geei*), a colobine primate endemic to Indo-Bhutan border, was discovered in the late 1950s based on morphological differences with the capped langur (*T. pileatus*). It has been listed as endangered species in the IUCN Red List and Schedule-I species in Indian Wildlife Protection Act (1972). Golden langur is found in the sub-tropical, monsoon-fed, semi-evergreen and mixed deciduous forests of western Assam in India and south-central Bhutan. It is considered to be one of the most restricted-range primates of South Asia. Habitat fragmentation consistently has large, negative effects on measures of biodiversity such as population size, distribution and genetic diversity, especially in habitat specialist species. The present study aimed to estimate the genetic diversity in the Indian populations of golden langur using HVRI. It also aims to examine the phylogenetic position of Indian golden langur with respect to the Bhutanese population and closely related *Trachypithecus* spp. using *cytb* (Fig 1). It is hoped that this study would serve as a platform for a more scientific approach towards managing the wild population of this enigmatic species.

Out of 71 samples collected, 59 samples gave successful amplification for HVRI and 518 bases were sequenced and aligned. Nineteen haplotypes were revealed based on 47 mutations (45 transitions and two transversions) spread over 46 segregating sites, out of which 43 were parsimony-informative. The overall haplotype diversity was high ($h = 0.938$) but the nucleotide diversity was low ($\pi = 0.02443$). The haplotypes were assigned an alphabetical code (from GL-A to GL-S) based on the chronology of discovery. Haplotype GL-C was the most frequent, represented by eight individuals from two fragments, followed by GL-A represented by seven individuals. More than half of the haplotypes represented fewer than three individuals. Haplotype and nucleotide diversities were calculated for each population. CS and BH showed the highest number of haplotypes (four each) and CR showed the second highest (three haplotypes). Consequently, CS and BH also had the highest haplotype diversities (0.76 and 0.8 respectively). Five fragments (KJ, NY, ND, MN and BG) showed two haplotypes while UM showed no variation and KJ, ND and BG showed the least number of polymorphic sites among their haplotypes. Surprisingly, NY, which had only two haplotypes, showed the most number of polymorphic sites (17) for any fragment, giving it the highest nucleotide diversity. It was followed by CR (14), CS (7), MN (7)

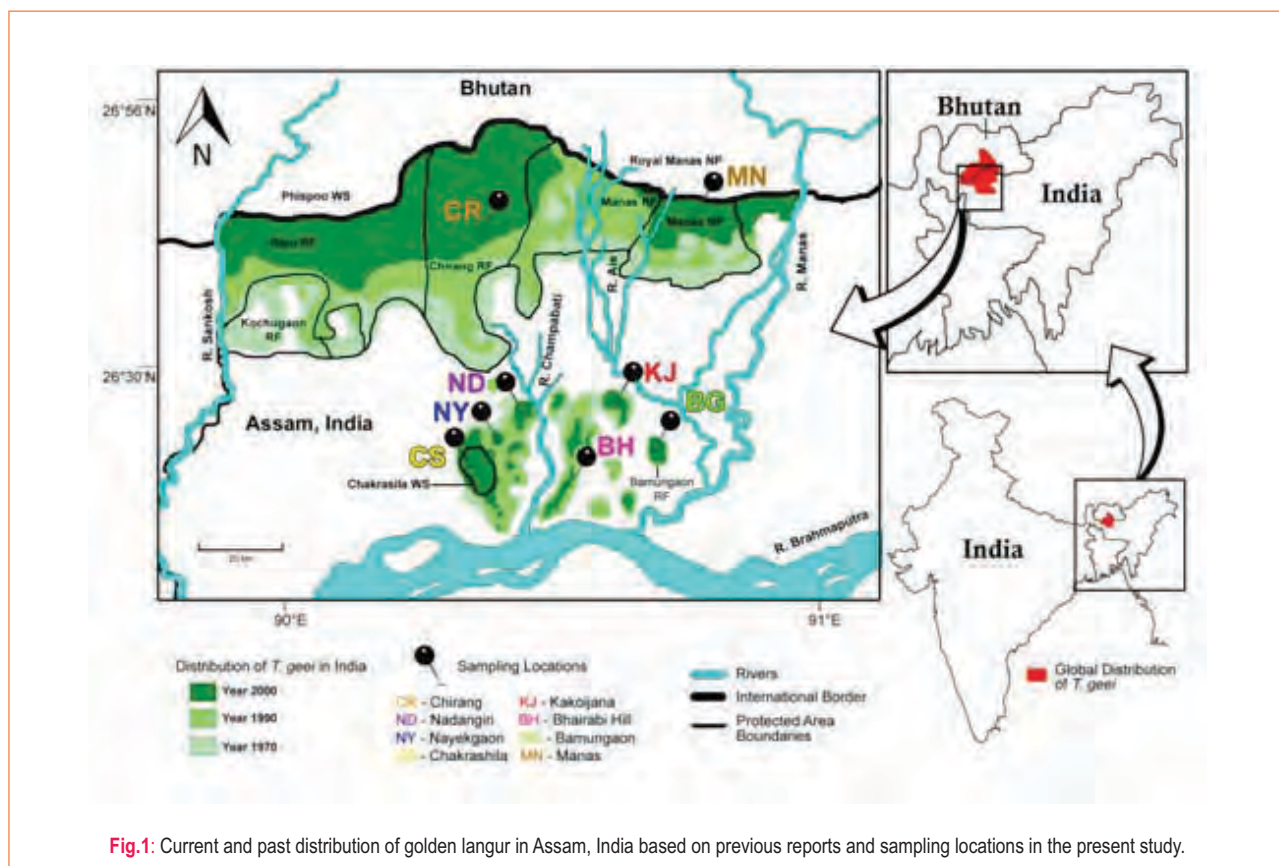
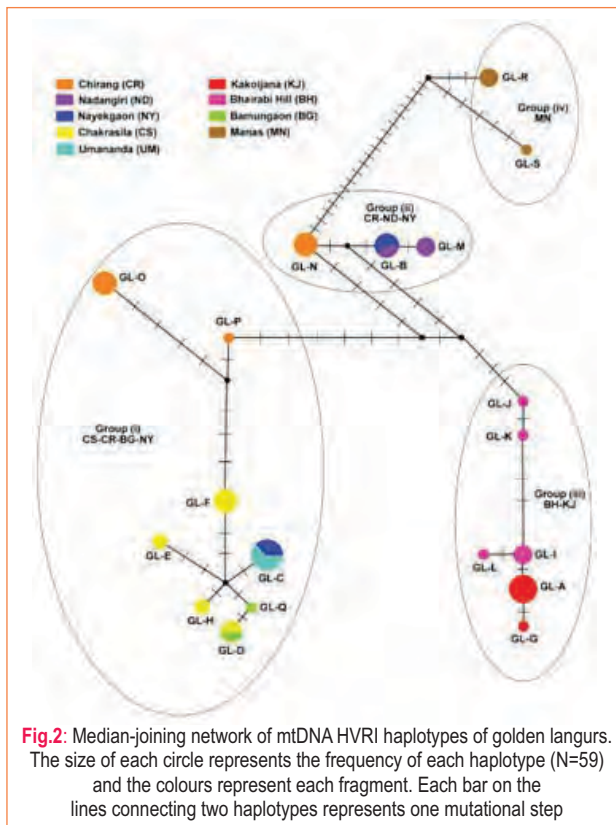
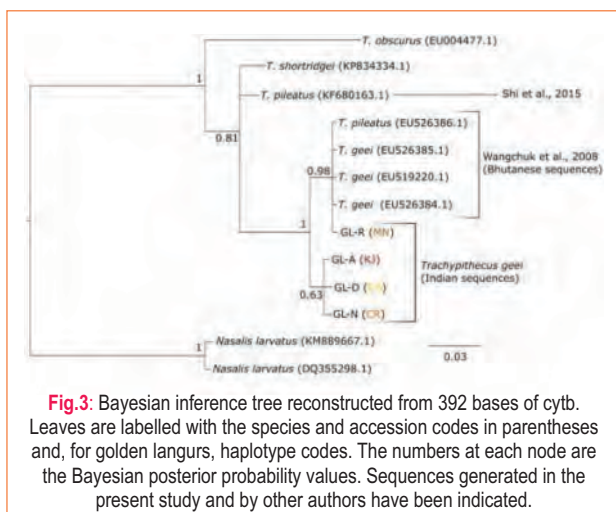


Fig.1: Current and past distribution of golden langur in Assam, India based on previous reports and sampling locations in the present study.



and BH (6). KJ had the lowest nucleotide diversity ($\pi = 0.000483$).

A median-joining haplotype network was drawn based on the HVRI sequence data (Fig 2). The 19 haplotypes differed from each other by between 1 and 42 bases. Three out of the 19 haplotypes were shared between utmost two fragments- haplotype GL-B was shared between NY and ND, GL-C was shared between NY and UM, and GL-D was shared between CS and BG. The haplotype network revealed no clear-cut structure, but there are at least four distinct groups- (i) all CS haplotypes along with two out of the three CR haplotypes and two other haplotypes from BG and NY/UM, (ii) Two ND haplotypes and the



remaining CR haplotype, (iii) all BH and KJ haplotypes and (iv) all MN haplotypes (Fig 2).

To check for the taxonomic placement of *T. geei*, a dataset containing 392 bases of partial cytb sequences was used in phylogenetic tree reconstruction. *T. geei*, *T. pileatus* and *T. shortridgei* clustered together with very high support, as expected, but the relationship among the three remained unresolved (Fig 3). Our sequences of *T. geei* clustered with the three sequences of Bhutanese *T. geei*. The haplotype GL-R from Manas had the same sequence as the Bhutanese *T. geei*. Curiously, one of the *T. pileatus* sequences (sampled from Bhutan also clustered with this *T. geei* clade (posterior probability of 1), whereas the other *T. pileatus* sequence separated out.

Although a majority of the fragments showed moderate to high genetic diversity for small, isolated populations, some fragments showed very low genetic diversity, particularly Kakoijana which has a population of about 144 golden langurs. Most forest fragments lost their connectivity between 1970s and 1990s. These fragments should be protected from further degradation and steps may be taken to connect them to maintain genetic diversity. Golden langurs are monophyletic, and it appears that they are capable of hybridizing with capped langurs in the wild and Hanuman langurs in captivity. In fact, some of the individuals kept in Indian zoos may be hybrids. Therefore, the genetic affiliation of captive golden langurs has to be ascertained before they are used in captive breeding programs and reintroductions.

Non-invasive monitoring of reproductive and stress hormones in the endangered red panda (*Ailurus fulgens fulgens*)

The red Panda (*Ailurus fulgens fulgens*), an endemic species of temperate forests in the Himalayan region, is categorized endangered by IUCN Red List 2015 due to its declining population by 50% over three generation. It is also listed under Schedule I species of the Indian Wild Life (Protection) Act 1972. Red panda is mainly confined to the Himalayas in India, Nepal, Myanmar and southern China. In India, their distribution is restricted to the North Eastern parts (Northern West Bengal, Sikkim, Arunachal Pradesh and Meghalaya) and south of the Himalayas (Choudhury, 2001). The major threats to its survival include habitat loss and poaching for its fur, mostly in China and Myanmar.

The red panda is usually nocturnal and solitary in nature. It mainly feeds on bamboos which is its primary sustenance (mostly tender leaves, shoots) and it also sometimes feeds on small mammals,



An adult Red panda at Darjeeling Zoo

bird eggs, fruits and acorns. Understanding of reproductive physiology is very important for the successful captive breeding program. As a part of the conservation breeding program of the red panda, the present study aimed to validate and standardize enzyme immunoassays (EIAs) to assess the fertility status, pregnancy detection and stress level in captive red panda using faecal steroid hormones. Faecal samples of females (n=4) and male (n=1) were collected every day in the morning between 8 to 9 hours from January 2013 to April 2014 at Padmaja Naidu Himalayan Zoological Park, Darjeeling, West Bengal, India. Faecal progesterone, testosterone and cortisol were measured previously validated in big cats and ungulates.

A total of 1471 faecal samples were collected from four adult females and one adult male for 15 months. Of the four females, matings in three females were observed, and one female gave birth to a cub during the study period. Of the 4 females monitored, three were observed matings with males during January, of which one female gave birth to a cub in June (Fig.4A). On the basis of mating observation and parturition, the gestation period was estimated to be 150 days. Overall, faecal 5α -pregnan- 3α -ol-20-one concentrations ranged widely from 370 ng/g (Sambridhi, a non pregnant animal) to 6000 ng/g (Sheetal, a pregnant animal). In non-pregnant animals, weekly mean basal faecal 5α -pregnan- 3α -ol-20-one concentration ranged from 370 ng/g to 3886 ng/g but it did not significantly vary among individuals (Friedman test $\chi^2 = 5.5$; $P = 0.067$). In the pregnant animal, it ranged from 2143 ng/g to 6000 ng/g. Following matings (Sheetal), the faecal 5α -pregnan- 3α -ol-20-one level raised gradually to 2 to 3 times higher than the basal values (mean = 1840; $n = 1016$) and the mean weekly concentrations increased significantly from 8 weeks until parturition (Friedman test $\chi^2 = 4.5$; $P = 0.03$) and decreased to basal concentration a week after parturition. Faecal cortisol levels were measured for all animals. Monthly mean concentrations ranged from 4.4 ± 0.81 ng/g to 107.5 ± 24.9 ng/g (Fig. 1). Both the lowest and highest values were observed in females. Overall, monthly mean cortisol concentrations were increasing with decreasing ambient temperature in all animals ($r_s = -0.874$; $P = 0.001$).

This study provides faecal 5α -pregnan- 3α -ol-20-one, testosterone and cortisol profiles in the captive red panda. EIA developed against progesterone metabolite could be used for reproductive monitoring and detection of pregnancy in the red panda. Furthermore, the study would facilitate reproductive monitoring of the red panda in captive breeding programs in India and elsewhere.

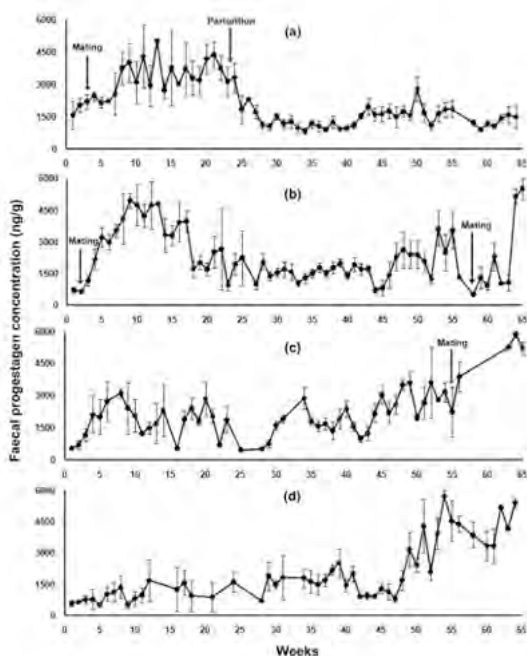


Fig.4: Faecal progesterone profiles of the four female red panda individuals (a-sheetal, b-Janaki, c-Sambridhi, d-Smile) - arrows indicate parturition and mating.

Ajay Gaur

Conservation Genetics of Endangered Species of India



From left to right: Vaishnavi Kunteepuram, Siuli Mitra, Ajay Gaur, B. Charan Kumar, A. Sreenivasu, Drishti R. Parmar

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

1. Tabasum W, Sreenivas A, Kesav Kumar B, Rao TG and Gaur A (2016) Complete mitochondrial genome sequence of Indian clouded leopard (*Neofelis nebulosa*). *Mitochondrial DNA*: doi: 10.1080/23802359.2016.1214543.
2. Tabasum W, Sreenivas A, Rai N, Thangaraj K and Gaur A (2016) Complete mitochondrial genome sequence of Asiatic lion (*Panthera leo persica*). *Mitochondrial DNA*: doi: 10.1080/23802359.2016.1214541.
3. Modi N, Sreenivas A, Tabasum W and Gaur A (2016) DNA tools to establish species and individual identity of confiscated biological samples in wildlife related crime: A case report. *Journal of Forensic, Legal and Investigative Sciences* 2: 006.
4. Ram M S, Marne M, Gaur A, Kumara H N, Singh M, Kumar A and Umapathy G (2015) Pre-Historic and recent vicariance events shape genetic structure and diversity in endangered Lion-tailed macaque in the Western Ghats: Implications for conservation. *PLoS ONE* 10: e0142597.
5. Gupta S, Kumar A, Gaur A and Hussain SA (2015) Detection of 40 bp insertion-deletion (INDEL) in mitochondrial control region among sambar (*Rusa unicolor*) populations in India. *BMC Res Notes* 8:581.

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 the genetic assessment of fragmented populations, resolution of taxonomic discrepancies and the use of molecular tools in forensics and understanding biology of endangered species. Our 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.

Evolutionary studies in the Asiatic lion

The Asiatic lion (*Panthera leopersica*) is classified as a member of Felidae family consisting of two major sub families Pantherinae and Felinae. Pantherinae includes six big cats i.e.: Tiger, Lion, Jaguar, Leopard, Snow leopard and Clouded leopard. Asiatic lions are the top carnivores and possess a prominent position atop the food chain in their only remaining natural habitat in Gir Forest of Gujarat in India - a reduction in their population size would therefore lead to an ecological imbalance. The Asiatic lion is listed as an endangered species by IUCN red list of threatened species.

We have sequenced and characterised the complete mitochondrial genome of the Asiatic lion. The overall mitogenome characteristics of the Asiatic lion were identical to a typical vertebrate mitochondrial genome. The complete mitochondrial genome of Asiatic lion is 17,059 bp in length. The gene order and origin of reading frame of all protein coding genes were identical to other members of Carnivora except for ND6 and nine tRNA genes. The total length of the 13 protein coding genes was 11,364 bp, which corresponds to 66.62% of the mitochondrial genome

sequence length. The longest gene was ND5 (1805 bp) and the shortest gene was ATP8 (195 bp). The length of the 22 tRNA genes ranges from 58 to 74 bp in Asiatic lion. All of the tRNAs can be folded into typical cloverleaf secondary structures, with the known exception of tRNA-Ser. The origin of L-strand replication (OL) was within a cluster of five tRNA genes i.e.: tRNA^{Trp}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys} and tRNA^{Tyr}. The noncoding region i.e. CR, was located between the tRNA-Pro and tRNA-Phe genes and is 1624 bp long. The alignment of the Felidae species control regions exhibited high genetic variability and rich A + T content. The characterization of Asiatic lion mitogenome will contribute to more refined phylogeny of big cats. Our study will also facilitate concentrated efforts towards the conservation and proper management of the only remaining population of Asiatic lions in India.

Conservation Breeding

Captive populations may provide individuals for reintroduction, whose success depends on off-setting inbreeding depression, loss of genetic diversity and genetic adaptation to captivity. With advances in conservation genetics it is now possible to clearly demarcate founder members and estimate numbers required to maintain a viable founding population in captivity. Later as the population approaches its target size, genetic issues in management like minimizing inbreeding & consequent inbreeding depression, and retaining genetic diversity can be addressed. The accumulation of deleterious mutations and genetic adaptation to captivity can be avoided so as to improve the success rate of reintroduction programs of the captive bred species into the wild.

The Central Zoo Authority (CZA) has initiated a conservation breeding programme, under which 23 endangered species have been identified and an effort has been initiated to identify and maintain a founder population for each of these species in

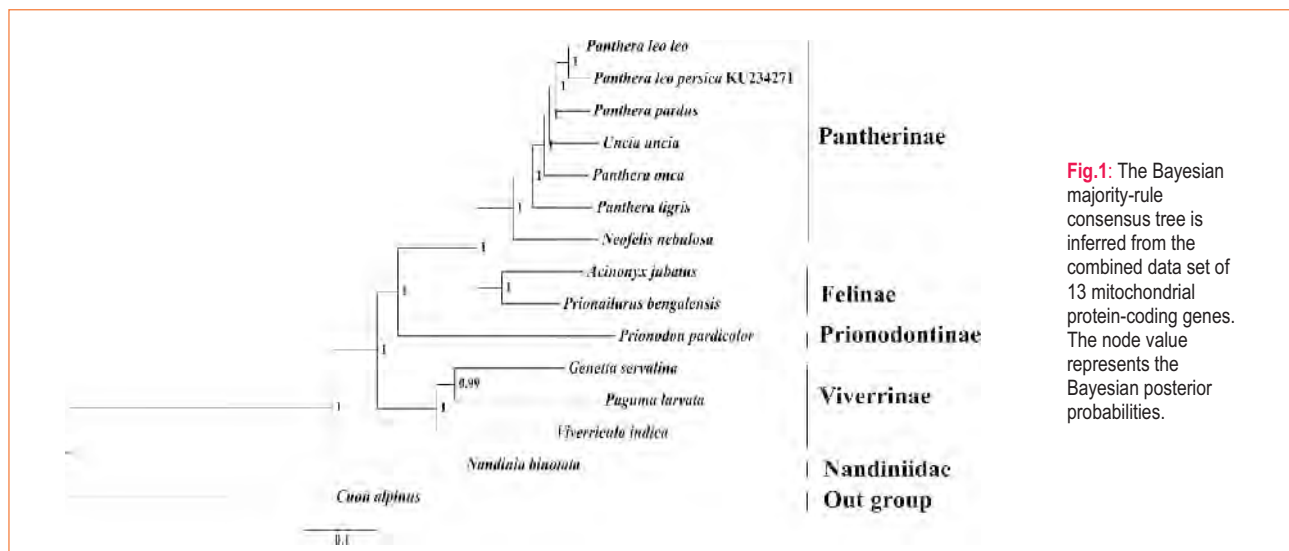


Fig.1: The Bayesian majority-rule consensus tree is inferred from the combined data set of 13 mitochondrial protein-coding genes. The node value represents the Bayesian posterior probabilities.

captivity. LaCONES is an important partner for this initiative and is conducting genetic analysis for the origin, purity and heterozygosity status. This work involves identification or development of nuclear microsatellite and mitochondrial markers. A large number of blood, tissue and feather samples of different species have been received under different species' conservation breeding programs for genetic health checkup. Six reports were submitted to respective Zoos and the CZA, for genetic health status, during last year (April 2016 – March 2017).

• Genetic studies in Asiatic lions

Genomic DNA was extracted from the blood samples of Eighteen (18) Asiatic lion housed in Lion Safari of Nehru Zoological Park, Hyderabad. The genetic status of the individuals was assessed in terms of their being heterozygous/homozygous at each locus. Further, DNA was amplified to generate molecular signatures (nucleotide sequences) from the mitochondrial cytb gene. Genotypes (Allelic distribution) of 18 Asiatic Lion samples for eight microsatellite loci were included for further analysis. The mean observed heterozygosity (H_o) was 0.472 and the mean expected heterozygosity was 0.439. Further, the analysis of the molecular signature (mtDNA cytb) generated from the above samples revealed four different haplotypes. The animals namely Ajay, Akash, Rita, Atul, Anthony, Aruna, Soniya and Jyothi shared similar haplotypes (H1), Sindhu, Sahiti, Jeetu, Vishwas, and Shilpa shared similar haplotypes (H2) and Vicky, Kashyap, Shruthi, and Vasu shared similar haplotypes (H3). Crazy was found to be separate haplotype (H4) and genetically most divergent lion among all the samples. The

animals found to be most heterozygous and unrelated were recommended to be used for selective breeding purpose.

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. It is one of the major on-going activities in LaCONES. We receive biological specimens confiscated in wildlife related crimes forwarded by the state forest, judiciary, police and customs departments. During the period April 2016 - March 2017, we have registered a total of 172 wildlife crime cases, out of which, 148 cases have been successfully reported to the forwarding authorities. During this period, a revenue of more than 15 lakhs was generated from the fee charges for DNA analysis. The forwarded biological samples comprised of 446 varieties such as meat, cooked/raw meat, skin, venom, tusks, bones, claws, feces, blood stains, saliva and swabs; we also recently received a large number of Hatha Jodi samples (Fig. 2).

Monitor Lizard (*Varanus bengalensis*) hemi-penis or the male sexual organs of monitor lizards are being sold in markets as holy plant root named as 'Hatha Jodi'. These lizards are being illegally poached from the wild, caught in traps and snares. The dried hemi-penis of monitor lizards looks like a dried plant root. As per traditional belief it is primarily sought as a tantric (black magic) piece for influencing people, winning over enemies and also to bring wealth, bravery, happiness and good fortune. On the basis of the DNA analysis using Universal Primers, we could establish the correct identity of all the 28 Hatha Jodi samples.



Fig.2: Confiscated Hatha Jodi samples

Krishnan H Harshan

Regulation of Protein Translation in Viral Infections and Biological Systems



From left to right: Mohan Singh Moodu, Manish Johri, Divya Gupta, Haripriya Parthasarathy, Hitha Nair, Dhiviya Vedagiri, Iqra Kuchay, Krishnan H Harshan

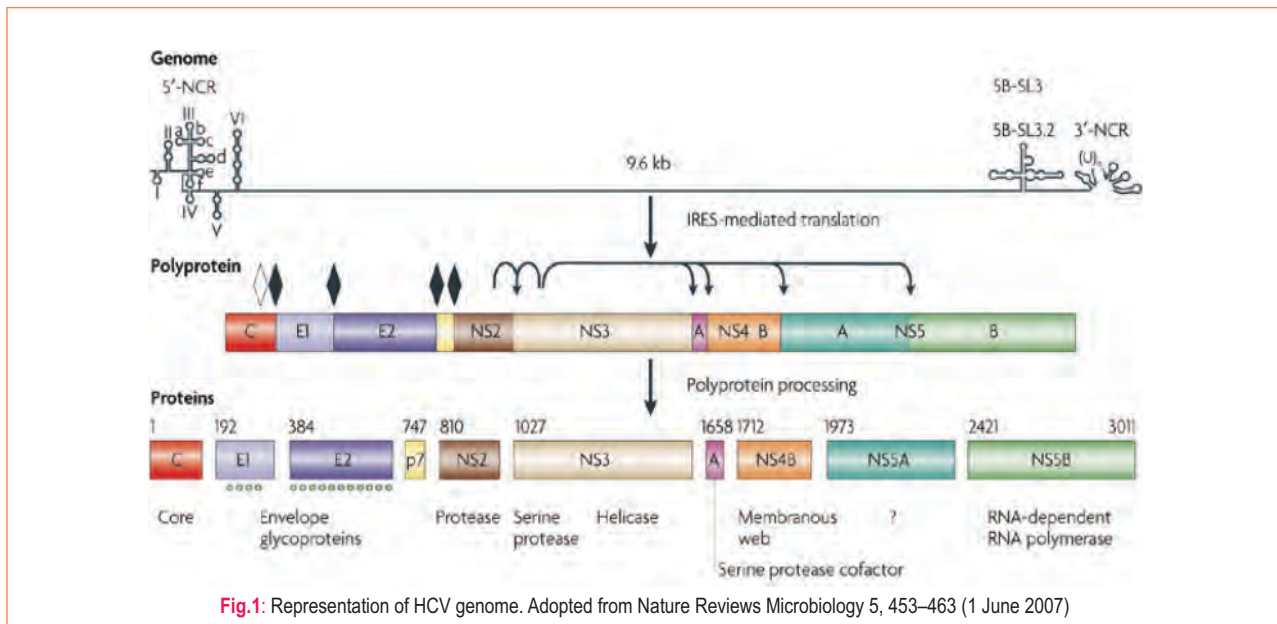
RESEARCH INTERESTS:

- Regulation of Protein Translation in Viral Infections
- Reprogramming of Protein Translation in Epithelial to Mesenchymal Transition

Oncoviruses establish a long-term relationship with hosts during chronic infections. They remain in a latent stage with minimal expression of viral proteins that helps them evade the host immune system. Our goal is to understand the manipulation of host translation by viral proteins and to understand its consequence on viral immune evasion and tumorigenesis.

Selected recent publications

1. Krishnan HH, Naranatt PP, Smith MS, Zeng L, Bloomer C, and Chandran B (2004) Concurrent expression of latent and a limited number of lytic genes with immune modulation and anti-apoptotic functions by KSHV/HHV-8 early during infection of primary endothelial and fibroblast cells and subsequent decline of lytic gene expression. *J. Virology*: 78:3601-20.
2. Krishnan HH, Walia N-S, Zeng L, Gao S J and Chandran B (2005) Envelope glycoprotein gB of Kaposi's sarcoma Associated Herpesvirus (KSHV/HHV-8) is essential for egress from infected cells *J. Virology* 79: 10952-10967.
3. Krishnan HH, Walia N-S, Streblov DN, Naranatt PP and Chandran B (2005) Focal Adhesion Kinase (FAK) is critical for Kaposi's Sarcoma Associated Herpesvirus (KSHV/HHV-8) entry into the target cells. *J. Virology*. 80:1167-1180. (Selected in the *J. Virology* spotlight in the same issue).
4. George A, Panda S, Kudmulwar D, Chhatbar S D, Nayak S C and Krishnan H H (2012) Hepatitis C virus NS5A binds to the mRNA Cap binding eIF4F complex and upregulates host translation initiation machinery through 4EBP1 inactivation. *J. Biol. Chemistry*. 10; 287(7): 5042-58.
5. Panda S, Vedagiri D, Viveka T S, Jonddula R C and Harshan K H (2014) Hepatitis C Virus Protein NS5A Coordinates a Unique Phosphorylation Dependent eIF4E Assembly on 40S Ribosomes that Regulates IRES Activity. *Biochem. J.* 462(2):291-302.



The role of deregulated host transcription in pathogenesis associated with viral infection is well acknowledged. However, the involvement of translation as a major regulatory event in various cellular activities including cell cycle regulation has only been recognized in the recent decade and a half. Of late, its involvement in tumor development and progression has been demonstrated unambiguously, suggesting the role of protein translation as major regulatory step in tumorigenesis. Translation is also exploited by many viruses for their preferential survival over the host. The focus of our research is to understand the involvement of translation in the pathogenesis of oncoviral infections.

In eukaryotes, the majority of the mRNA transcripts are translated by 5'Cap initiation, which starts with the binding of eukaryotic translation initiation factor 4F complex (4F) to the cap structure. The 4F complex is composed of three different eukaryotic translation initiation factors, eIF4E (4E), eIF4G (4G) and eIF4A (4A). 4F complex formation is a major step in translation initiation and is the dearest rate-limiting step in the process. Under phosphorylated 4E binding proteins 4EBP-1 and 4EBP-2 competitively bind to 4E, thus inhibiting its interactions with 4G and subsequent 4F complex formation. This results in the inhibition of cap mediated translation rate. This block can be removed by the hyper phosphorylation of 4EBPs by mammalian target of rapamycin (mTOR). mTOR is phosphorylated by AKT and is negatively regulated by phosphatase and tensin homolog (PTEN) and tuberous sclerosis proteins (TSC1 and 2). mTOR also phosphorylates p70 S6 Kinase (S6K), which positively regulates translation initiation along with 4EBP phosphorylation.

HCV and host translation

Microbiology 5, 453–463 (1 June 2007) | doi:10.1038/nrmicro1645 Hepatitis C Virus (HCV) is a member of *flaviviridae* family and has a single stranded RNA genome of about 9.6 kb with positive polarity. It is a major causative agent of liver cirrhosis (LC) and hepatocellular carcinoma (HCC) in humans. About 1-3% of the worldwide population is estimated to be infected with HCV. Currently, there are no vaccines against HCV infection. The combinational therapy of interferon- α and antiviral drug ribavirin is used to treat patients with varied success that depends on the HCV genotype. The RNA genome of HCV undergoes mutations at a very high frequency, making it very difficult to target the virus with drugs. HCV has an internal ribosome entry site (IRES) in its 5'untranslated region (5'UTR), which it uses for its translation. Unlike 5'Cap mediated translation, IRES mediated translation is significantly less dependent on various host factors and can still operate in the event of host translation shut off. Many viruses shut off host translation in various ways to hijack the machinery for viral translation, even as the degree of host translation shut off could vary and hence is a deciding factor in pathogenesis. While many viruses still manage to keep the host cell alive for their own survival, some virus infections lead to total host translation shut off resulting in its death and subsequent termination of viral cycle progression. HCV allows host translation to go on as is evident from the nature of infection. The virus establishes a long term chronic infection in hepatocytes and infected people develop symptoms only after 10-15 years post-infection.

Our laboratory seeks to understand various signaling pathways/molecules regulated by HCV proteins in human liver cells by using two different systems:

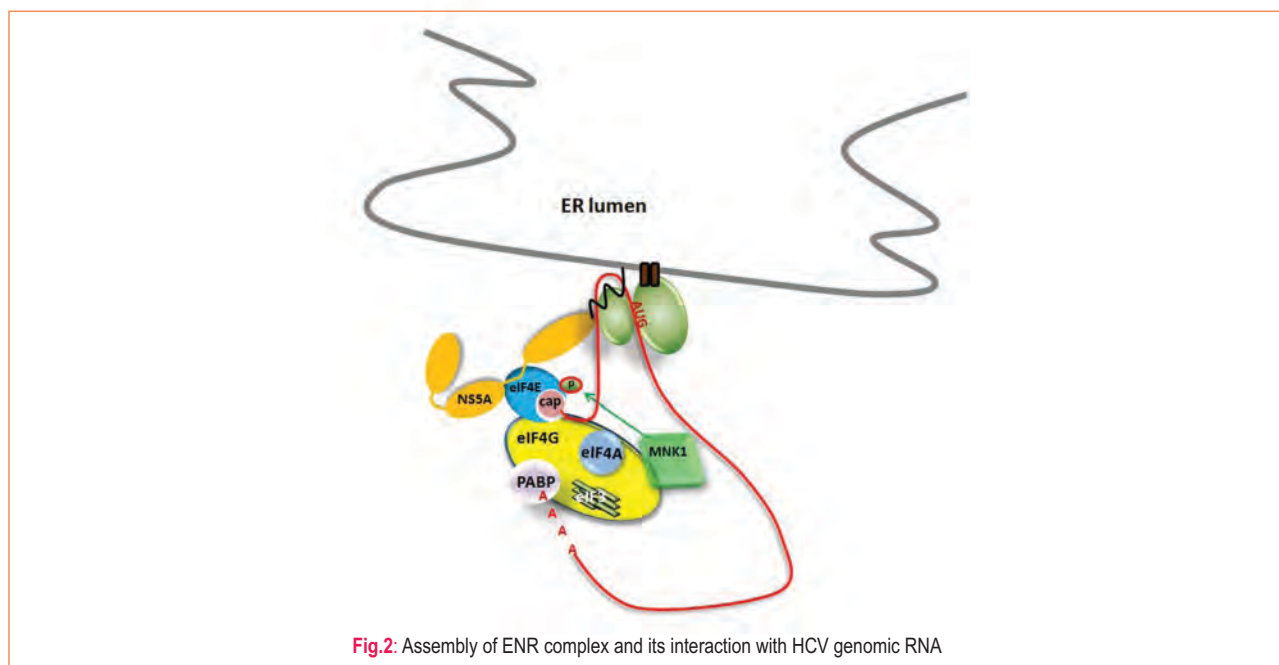
- a. Infection of hepatocytes by cell culture prepared infectious HCV clones (HCVcc). HCVcc is prepared in a biosafety level -2 (BSL-2) and human hepatoma and primary hepatocytes are infected in the containment facility.
- b. Over-expression of HCV proteins in hepatocytes. Effects of both HCVcc infection and viral protein expressions are being studied using various biochemical, cellular and molecular biological methods. Infected and HCV protein over-expressing cells are used to prepare protein lysates to study various signaling events leading to regulation of translation initiation. We employ microscopy, flow cytometry and microarray based analyses to understand the implication of translation in HCV associated pathogenesis in detail.

Our earlier studies showed that HCV enhances cap-dependent translation in Huh7.5 cells through activation of mTORC1 and also by phosphorylating eIF4E. Subsequent studies on regulation of translation by HCV NS5A demonstrated a direct association of NS5A with 40S ribosomes through eIF4E leading to formation of a unique complex ENR (eIF4E-NS5A-Ribosome). Further studies with bicistronic luciferase vectors carrying HCV IRES identified that these regions of domain 1 are also critical in the upregulation of IRES by NS5A, directly

linking the ENR assembly to HCV pathogenesis. We are currently trying to understand the contribution of activated mTORC1 complex and eIF4E to the whole process of viral life cycle.

Protein Translation in Epithelial Mesenchymal Transition

Rapidly proliferating tumor cells demand larger amounts of proteins. If protein translation rates are not synchronized with that of transcription, this demand cannot be met. Tumor cells meet this demand by increasing ribosomal biogenesis and upregulating protein translation machinery. Epithelial mesenchymal transition (EMT) is a process by which epithelial cells abandon many of their characteristic features and acquire various mesenchymal ones. During this process, epithelial cells lose their adhesive nature, characteristic extracellular matrix sheath and polarity while acquiring spindle shape with tapering ends and migratory capabilities. EMT is demonstrated to be behind the assimilation of metastatic capabilities by tumor cells. This transition facilitates increased migratory and invasive capabilities of tumor cells. We have characterized an atypical EMT in human hepatoma cells and identified substantial upregulation of protein translation initiation during this process. We identified that GSK-3 β is critical in maintaining the mesenchymal phenotype and that p38MAPK and ERK1/2, two major MAPKs, regulate this activity through GSK-3 β . Our laboratory is actively researching the link between protein translation regulation and EMT.



K Guruprasad

Protein Sequence, Structure Analysis and Drug Design



Pallabini, Guruprasad, Upasana

RESEARCH INTERESTS:

- Bioinformatics
- Protein sequence/structure analysis
- Protein structure prediction & three-dimensional modeling
- Protein function prediction
- Drug-design

The research in our group encompasses the following: exploring protein sequence-structure relationships, predicting the structure and function of proteins of interest, predicting potential therapeutic targets and drugs in human disease, development of methods, software and databases relevant to bioinformatics.

Selected recent publications

1. Sridhar S, Pallabini D, Guruprasad K (2016) Comparative analyses of the proteins from *Mycobacterium tuberculosis* and human genomes: Identification of potential tuberculosis drug targets. *Gene* 579(1):69-74 [Also see, NATURE INDIA (18th January 2016), <http://www.natureasia.com/en/nindia/article/10.1038/nindia.2016.5> & India Bioscience (16th Feb 2016), <https://indiabioscience.org/news/2016/a-bioinformatics-based-approach-to-identify-targets-for-potential-drug-treatment-of-tuberculosis>].
2. Gurpreet K, Guruprasad K, Brenda R S Temple, David G S, Nikolay V D, Pratap K P (2017). Structural complexity and functional diversity of the plant NADPH oxidases. *Amino Acids* (in press).
3. Pallabini D, Bala D M, Lalitha G and Guruprasad K (2017) Three-dimensional models of *M. tuberculosis* proteins Rv1555, Rv1554 and their docking analyses with sildenafil, tadalafil, vardenafil drugs, suggest interference with quinol binding likely to affect the protein's function (manuscript undergoing minor revision, BMC Structural Biology, 2017).

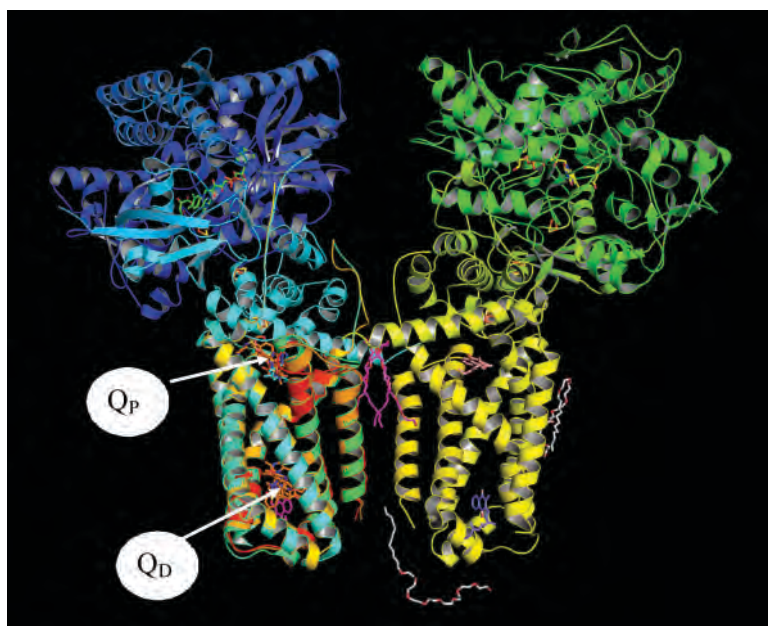


Fig.1: Structural overlay of the *M.tb* monomer (Rv1555) and *M.tb* dimer (Rv1555, Rv1554) proteins complexed with sildenafil, tadalafil, vardenafil drugs on to the crystal structure of *E.coli* QFR tetrameric dimer protein complex (PDB code: 1L0V). The drug binding sites coincides with the equivalent menaquinol binding at the Q_D site for monomer and at the Q_P site for dimer *M.tb* proteins.

Prediction of the inhibitory action of potential repurposed drugs against novel *M.tb* targets

We recently identified 94 new potential TB drug targets based on a bioinformatics approach developed in our laboratory. Ten of these targets were also predicted to be 'druggable' with certain known drugs in the market (GENE 2016). During the present year, we have analyzed one of the potential druggable targets, in order to understand the possible mechanism by which the protein's function is likely to be inhibited. Our analyses based on molecular modeling and docking suggests that the *M. tuberculosis* Rv1555 and Rv1554 proteins are likely to be inhibited by some of the known drugs; sildenafil (Viagra), tadalafil (Cialis) and vardenafil (Levitra) drugs, by interfering with the quinol binding sites essential for the protein's function as observed in the homologous *E.coli* quinol fumarate reductase respiratory protein complex. Further, via collaboration

with School of Chemistry, University of Hyderabad, we have observed the inhibition of *in-vitro* growth of *E.coli* bacteria containing the homologous *M.tb* proteins with sildenafil citrate and tadalafil drugs. Our analyses suggest dual binding sites, Q_D (distal) and Q_P (proximal) for the monomer and dimer *M.tb* proteins, respectively. The predicted binding sites corroborate with the experimentally observed binding sites in the *E.coli* fumarate reductase protein crystal structure complex. We therefore suggest that sildenafil citrate and related drugs currently used in the treatment of male erectile dysfunction targeting the human phosphodiesterase 5 enzyme may be evaluated for their plausible role as repurposed drugs in the treatment of tuberculosis.

We are currently analyzing the possible inhibitory action of the other drugs and their potential new targets predicted and reported by us (GENE 2016).

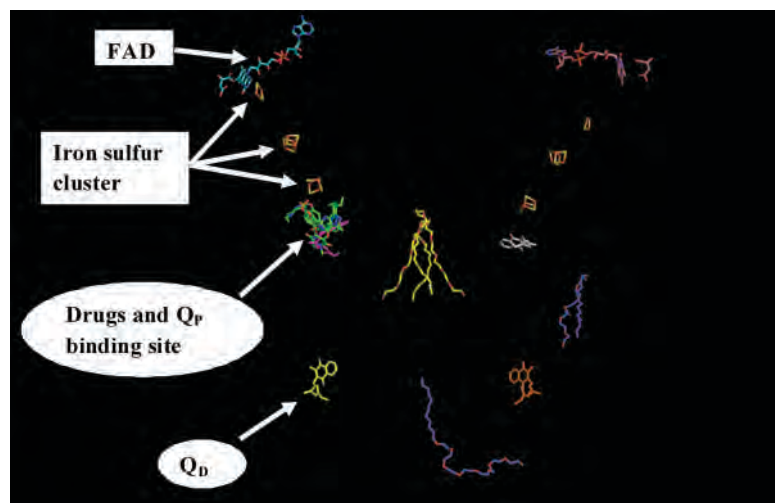


Fig.2: The FAD, iron-sulfur clusters, menaquinol molecules bound near the Q_P and Q_D sites in the crystal structure of *E.coli* QFR tetrameric dimer protein complex (PDB code: 1L0V) (proteins excluded for the sake of clarity). Sildenafil, tadalafil, vardenafil drug binding coinciding with the Q_P site in the *M.tb* dimer protein.

K Thangaraj

Evolutionary and Medical Genetics



From left to right & Top to bottom: Umesh Kumar, Rajan K. Jha, Vishwanath, Satya Ranjan Singh, Lomous Kumar, Niraj Rai, Jaydeep A. Badarukhiya, A. Paramasivam, Mohd. Haneef, Sheikh Nizamuddin, Jagamohan Chhatai, D V S Sudhakar, Sunil K. Tripathi, Venkatapathi Challa, Deepak K. Kashyap, G. Mala, Sunitha Kundur, Usha Kabilan, Nipa Basak, Narmadha Ganapathy, S Deepa Selvi Rani, K. Thangaraj

RESEARCH INTERESTS:

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

We have demonstrated that how the 1.5 billion people of South Asia are vulnerable to rare and population-specific diseases, due to practice of endogamy for the last 2000 years.

Selected recent publications

1. Mallick S, Thangaraj K, Pääbo S, Kelso J, Patterson N, Reich D (2016) The Simons Genome Diversity Project: 300 genomes from 142 diverse populations. *Nature*. 538: 201-206.
2. Mishra A, Nizamuddin S, Mallick CB, Singh S, Prakash S, Siddiqui NA, Rai N, Carlus SJ, Sudhakar DV, Tripathi VP, Möls M, Kim-Howard X, Dewangan H, Mishra A, Reddy AG, Roy B, Pandey K, Chaubey G, Das P, Nath SK, Singh L, Thangaraj K (2017) Genotype-Phenotype Study of the Middle Gangetic Plain in India Shows Association of rs2470102 with Skin Pigmentation. *J Invest Dermatol*. 137:670-677.
3. Chaubey G, Tamang R, Pennarun E, Dubey P, Rai N, Upadhyay RK, Meena RP, Patel JR, van Driem G, Thangaraj K, Metspalu M, Villems R (2017) Reconstructing the population history of the largest tribe of India: the Dravidian speaking Gond. *Eur J Hum Genet*. 25:493-498.
4. 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 Biol*. 18:110.
5. 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. *Nat Genet*. 49: 1403-1407.

We have earlier demonstrated that the Indian populations are the descendents of the very first modern human migrated Out-of-Africa about 60,000 years ago. Since then most of the Indian populations are in isolation and follow endogamy marriage practice, at least for the last 2000 years. As a result, Indian populations acquired unique set of genetic variations, which are of Indian-specific and responsible for causing diseases in India. Therefore, our main research interest is to study the genetic variations among Indian populations to understand their origin, health and disease.

The promise of discovering population-specific disease-associated genes in South Asia

South Asia is inhabited by about 5,000 anthropologically well-defined populations, many of which are endogamous communities with significant barriers to gene flow due to sociological, linguistic and cultural factors that restrict inter-population marriage. To assess the impact of endogamy, we have analysed samples from more than 2,800 individuals from over 275 distinct South Asian groups from India, Pakistan, Nepal, Sri Lanka, and Bangladesh using about 600,000 genome-wide markers. We found that 81 out of 263 unique South Asian groups, including 14 groups with estimated census sizes of over a million, have a strong founder event than the one that occurred in both Finns and Ashkenazi Jews in the West – these are founder groups known to have large numbers of recessive diseases (Figure 1). We identified multiple examples of recessive diseases in South Asia that are the result of such founder events. Our study provides opportunity for discovering population-specific disease causing genes in communities known to have strong founder events. Mapping of mutations that are responsible for population-specific disease would help in developing strategies for diagnosis, counseling, management and modifying the clinical course of these disorders and to reduce the disease burden among South Asians.

Reconstructing the population history of the largest tribe of India: the Dravidian speaking Gond

The linguistic landscape of India is composed of four major language families and a number of language isolates and is largely associated with non-overlapping geographical divisions. The majority of the populations speak Indo-European languages, which cover a large geographical area including northern and western India. Dravidian languages are

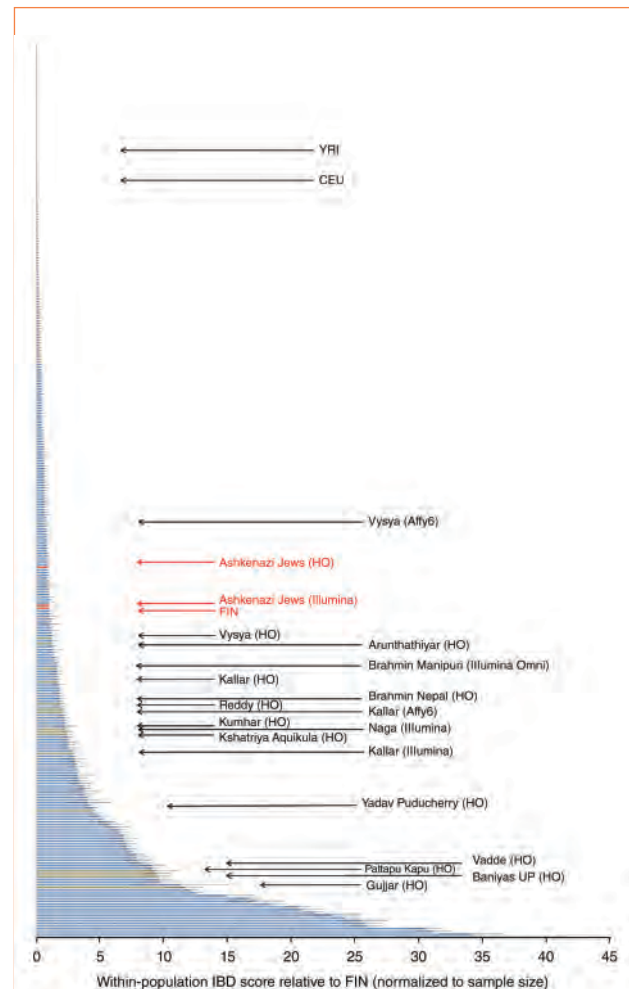


Fig.1: IBD scores relative to Finns (FIN). Histogram ordered by IBD score, which is approximately proportional to the per-individual risk for recessive disease due to the founder event. (These results are also given quantitatively for each group in Supplementary Table 5.) We restricted our analysis to groups with at least two samples, combining data from all four genotyping platforms into one plot. Data from Ashkenazi Jews and Finns are highlighted in red, and those from South Asian groups with significantly higher IBD scores than those of Finns and census sizes of more than 1 million are in brown. Error bars for each IBD score are standard errors calculated by weighted block jackknife over each chromosome. YRI, Yoruba (West African); CEU, European American. Data sets are indicated in parentheses: Affy6, Affymetrix 6.0 data; HO, Human Origins data.

spoken primarily in southern India with some exceptions, eg, Brahui in Pakistan, Kurukh–Malto in eastern India and Gond–Manda languages in central India. The Gond comprise the largest tribal group of India with a population exceeding 12 million. Linguistically, the Gond belongs to the Gond–Manda subgroup of the South Central branch of the Dravidian language family. Ethnographers, anthropologists and linguists entertain mutually incompatible hypotheses on their origin. Genetic studies of these people have thus far suffered from the low resolution of the genetic data or the limited number of samples. Therefore, to

gain a more comprehensive view on ancient ancestry and genetic affinities of the Gond with the neighbouring populations speaking Indo-European, Dravidian and Austroasiatic languages, we have studied four geographically distinct groups of Gond using high-resolution data. All the Gond groups share a common ancestry with a certain degree of isolation and differentiation. Our allele frequency and haplotype-based analyses reveal that the Gond share substantial genetic ancestry with the Indian Austroasiatic (ie, Munda) groups, rather than with the other Dravidian groups to whom they are most closely related linguistically (Figure 2).

The paternal ancestry of Uttarakhand does not imitate the classical caste system of India

Although, there have been rigorous research on the Indian caste system by several disciplines, it is still one of the controversial socio-scientific topic. Previous genetic studies on the subcontinent have supported a classical hierarchal sharing of genetic component by various castes of India. In the present study, we have used high-resolution mtDNA and Y chromosomal markers to characterize the genetic structuring of the Uttarakhand populations in the context of neighboring regions. Furthermore, we have

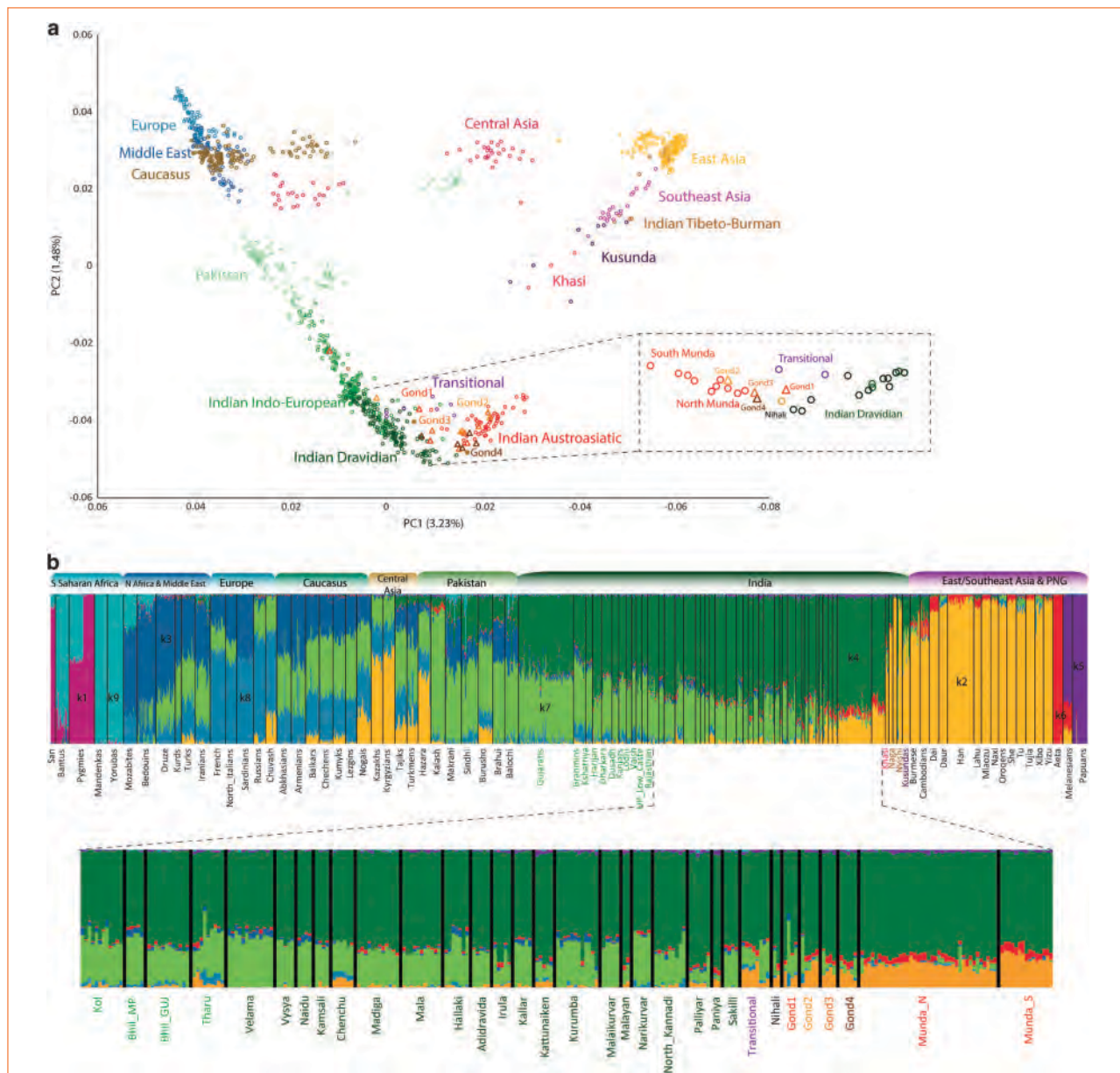


Fig.2: (a) Principal component analysis (PCA) of the combined autosomal SNP data of individuals from Eurasia. The inset picture showed the plot of mean eigen values of Gond and their genetic neighbours. (b) Plot of population-wise unsupervised ADMIXTURE analysis (K = 9) of world population with a zoom-in of various Indian populations including Gonds. The colour codes of the Indian populations have been given according to their linguistic affiliation shown in Figure 1a. Bhil_GUJ, Bhils from Gujarat; Bhil_MP, Bhils from Madhya Pradesh; Munda_N, North Munda group; Munda_S, South Munda group.

tested whether the genetic structuring of caste populations at different social levels of this region, follow the classical chaturvarna system. Interestingly, we found that this region showed a high level of variation for East Eurasian ancestry in both maternal and paternal lines of descent. Moreover, the intra-population comparison showed a high level of heterogeneity, likely because of different caste hierarchy, interpolated on asymmetric admixture of populations inhabiting on both sides of the Himalayas.

Leber's hereditary optic neuropathy-specific mutation m.11778G>A exists on diverse mitochondrial haplogroups in India

Leber's hereditary optic neuropathy (LHON; OMIM 535000) is one of the most common maternally inherited mitochondrial disorders. Three mitochondrial DNA point mutations—m.3460G>A (MT-ND1), m.11778G>A (MT-ND4), and m.14484T>C (MT-ND6)—account for the majority of reported LHON cases. Only approximately 50% of males and approximately 10% of females carrying these mutations develop optic neuropathy and blindness. Additional factors, such as mtDNA/nuclear genetic background and environmental modifiers, are likely to contribute toward the observed incomplete penetrance and gender bias. We aimed to investigate whether mtDNA haplogroup influences LHON clinical expression in Indian patients harboring the m.11778G>A mutation. Detailed clinical assessment and complete mitochondrial genome sequencing was undertaken in 64 LHON families harboring the m.11778G>A mutation. Mitochondrial haplogroup was assigned based on evolutionarily conserved mtDNA variations. A total of 543 individuals (295 male, 248 female) from 64 unrelated families harboring the m.11778G>A mutation were recruited to the study. The overall disease penetrance was 27.07% (146 of 543) and higher in males (37.9%; 112 of 295) than females (13.7%; 34 of 248). The mtDNA haplogroup analysis revealed that all affected probands belonged to different mtDNA haplogroups. No association between the m.11778G>A mutation and the background mtDNA haplogroup was

detected. The first detailed study of Indian LHON patients confirm that the m.11778G>A related LHON in India coexists with multiple different mtDNA haplogroups, unlike the preferential association of west Eurasian haplogroup J and the reported increased clinical penetrance with the J2 sub-haplogroup. However, we observed variable penetrance of LHON in different Indian mtDNA haplogroup backgrounds, indicating their possible influence on clinical expression. These data suggest that a similar heterogeneity, resulting from the mtDNA haplogroup, might also exist in other mitochondrial diseases among Indian populations.

Mutation in CETP is associated with coronary artery disease in south Indians

Coronary artery disease (CAD) is one of the leading causes of mortality worldwide. It is a multi-factorial disease and several studies have demonstrated that the genetic factors play a major role in CAD. Although variations in cholesteryl ester transfer protein (CETP) gene are reported to be associated with CAD, this gene has not been studied in South Indian populations. Hence, we evaluated the CETP gene variations in CAD patients of South Indian origin. We sequenced all the exons, exon-intron boundaries and UTRs of CETP in 323 CAD patients along with 300 ethnically and age matched controls. Variations observed in CETP were subjected to various statistical analyses. Our analysis revealed a total of 13 variations. Of these, one 3'UTR variant rs1801706 (c. *84G>A) was significantly associated with CAD (genotype association test: OR = 2.16, 95% CI: 1.50±3.10, p = 1.88×10⁻⁵ and allelic association test: OR = 1.92, 95% CI: 1.40±2.63, p = 2.57×10⁻⁵). Mutant allele ^aA^o was observed to influence the higher concentration of mRNA (p = 7.09×10⁻³, R² = 0.029 and β = 0.2163). Since expression of CETP has been shown to be positively correlated with the risk of CAD, higher frequency of ^aA^o allele (patients: 22.69% vs. controls: 13%) reveals that c.*84G>A is a risk factor for CAD in South Indians. This is the first report of the CETP gene among South Indians CAD patients. Our results suggest that rs1801706 (c.*84G>A) is a risk factor for CAD in South Indian population.

Lakshmi Rao Kandukuri
Chromosome Biology and Human Reproductive Genetics



From left to right: Praveen K, Naresh M, Padmalatha OV, Lakshmi Rao K, Kanakavalli MK, Amulya Madhuri K.

RESEARCH INTERESTS:

- Chromosomal and genetic evaluation of infertile men
- Genetics of reproductive dysfunction in women
- Understanding the genetic basis of recurrent early pregnancy loss
- Characterizing chromosome breakpoints associated with infertility

Selected recent publications

1. Aswini S, Kanakavalli, MK, Anuradha D, Chandra RS and Lakshmi Rao K (2016) Ring Chromosome 9 and Chromosome 9p Deletion Syndrome in a Patient Associated with Developmental Delay – A Case Report and Review of Literature, *Cytogenetics and Genome Research* 148(2-3):165-73.
2. Aswini S, Kanakavalli MK, VenkataPadmalatha O, Anuradha D, Chandra RS, Lakshmi Rao K (2017) De novo 'pure' partial trisomy (6)(p22.3→pter) - a case report and review of the literature, *Clinical Dysmorphology* 26(1):26-32.

Under the various human reprogenetics programmes, the group focuses on chromosomal and molecular basis of spermatogenesis, ovarian development and cycling disorders, polycystic ovarian syndrome and recurrent early pregnancy loss.

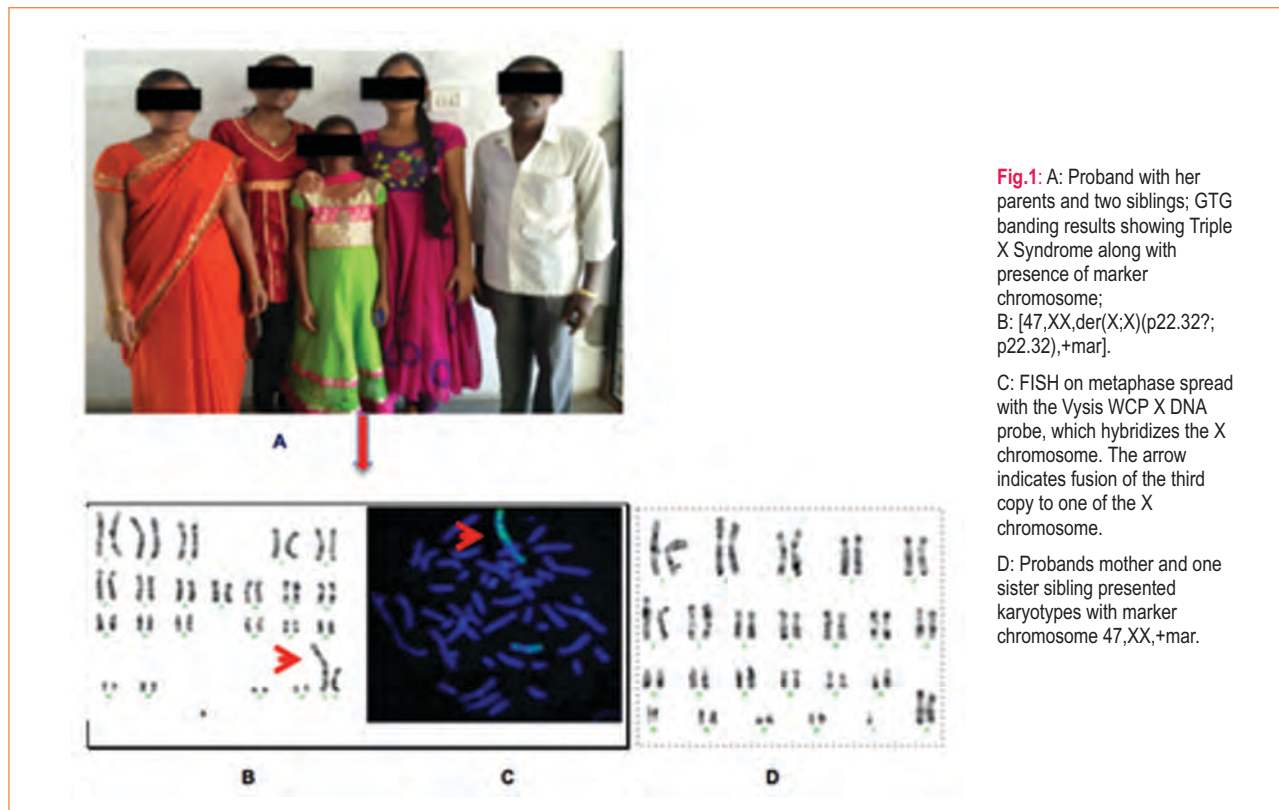
In addition to identifying chromosome defects, our group also undertakes research to understand the genetic etiology of human reproductive disorders. We also carry out chromosome diagnosis for patients with short stature, hypogonadism, undescended testis, microcephaly, delayed developmental milestones, familial and isolated mental retardation, and cerebral palsy.

Among the 77 postnatal referral cases, an 'unusual triple XXX syndrome with a marker chromosome associated with primary amenorrhea' is presented wherein the proband, aged 13 years, was referred for chromosome analysis with the diagnosis of primary amenorrhea. She was first seen as an outpatient in an endocrine clinic because of her short stature and primary amenorrhea. Pelvic ultrasonography showed small hypoplastic uterus and ovaries like structures in adnexae. The endocrinologic examination revealed elevated levels of serum FSH (126.6 mIU/mL; normal 3–20 mIU/mL) and normal LH levels. Skeletal maturity was abnormal for her age, and corresponded to an age of 10 years. The product of a first full-term pregnancy with normal delivery of a non-consanguineous parents, she has two healthy elder sisters. Her intellectual development was normal and allowed to pursue her academic activities.

Cytogenetically, this case with primary amenorrhea was analyzed with an abnormal karyotype [47,XX,der(X;X)(p22.32?;p22.32),+mar] analysed in 100 metaphases. Normally females have two X chromosomes with 46,XX karyotype. The proband shows triple X syndrome presenting three copies of X chromosome and one additional marker chromosome in all the 45 analyzed metaphases. Also observed the derivative X chromosome formed by fusion of third copy of the X chromosome giving rise to Robertsonian translocation as indicated by red arrows in the Fig 1A (GTG banding) and 1B (FISH using whole chromosome paint for chromosome X). The effects on the proband are exactly the same as Triple X syndrome associated having an extra entire X chromosome.

The proband's parents and two sibling sisters also were further investigated for chromosomes; wherein mother and one sibling sister showed one additional marker chromosome presented 47,XX,+mar karyotype in all the 20 analyzed metaphases which confirms its stability. Her father and other sister showed a normal karyotypes.

The unusual triple X karyotype as presented by the proband was further characterized; The presence of Centromeres, Androgen Receptor locus (Xq11-12), XIST locus (Xq13.2) and



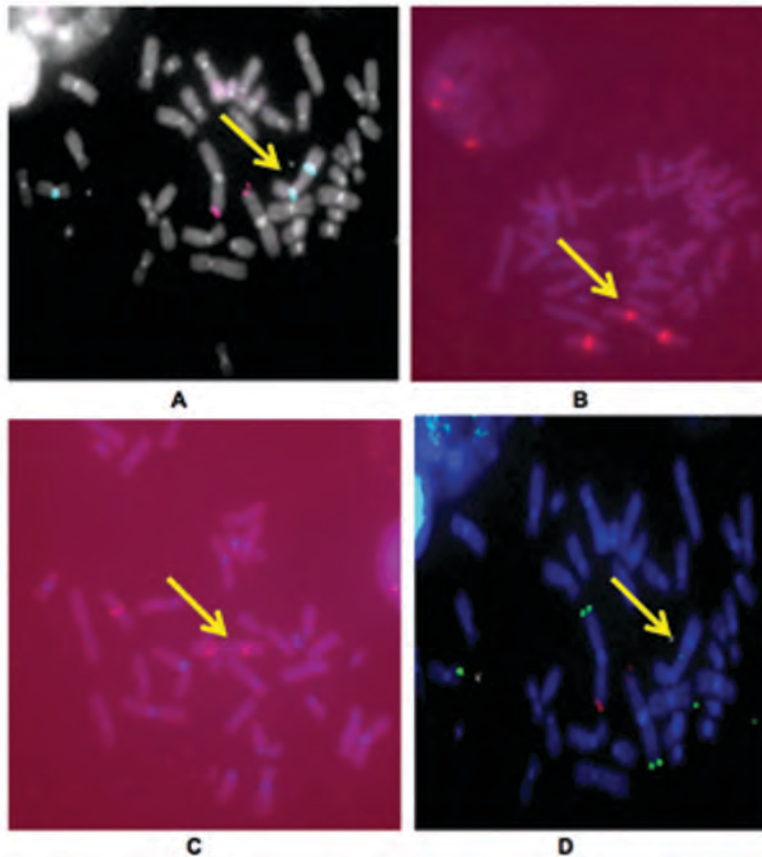


Fig.2: FISH on metaphase spread with the Vysis

A: CEP X DNA probe, which hybridizes the X centromere

B: Androgen Receptor DNA probe

C: XIST DNA probe, shows two signals on derivative chromosomes D: Xp (green) and Xq (red) subtelomere DNA probes showed Xp green signals as interstitially localized as indicated by arrow in each figure.

subtelomere regions of X of the X chromosome was confirmed by FISH using a locus-specific painting probe. The Centromeres, androgen receptor, XIST and subtelomere signals are seen in normal X and fusion forms of the X chromosome as shown in Fig 2: A, B, C and D. The derivative X chromosome presented two signals of Centromere, Androgen Receptor & Xist Locus, whereas in case subtelomere

signals of Xp regions are noted to be interstitially localized.

This novel X chromosome abnormality presented associated with ovarian defect and molecular cytogenetic characterization of derivative X chromosome would be helpful for genetic counseling and besides, infertility clinics could use them to decide suitable strategies to help such patients.

Arvind Kumar

Non-coding RNAs in Diverse Brain Regions in Stress Response and Depression



From left to right: V. Lenin, Sachin Singh, Arvind Kumar, Avijeet Kamle, Archana Golla, Unis Ahmad Bhat, Sandeep Kumar Dey, Shams-ul-Haq, Jitendra Kumar Sinha, Nitin Khandelwal

RESEARCH INTERESTS:

The focus of the lab is to unravel the epigenetic regulatory mechanisms in chronic neuropsychiatric diseases, in particular depression and related affective disorders.

Currently, the study on 3 of the lincRNAs is in progress using ex vivo and in vivo models.

Selected recent publications

1. Pathak SS et al. (2017) Histone lysine demethylases of JMJD2 or KDM4 family are important epigenetic regulators in reward circuitry in the etiopathology of depression. *Neuropsychopharm*, 42, 854-863.
2. Chakravarty S et al. (2017) Insights into the epigenetic mechanisms involving histone lysine methylation and demethylation in ischemia induced damage and repair has therapeutic implication. *BBA - Molecular Basis of Disease*, 1863:152-164.
3. Joshi PC et al (2017) A 2-oxa-spiro[5.4] decane scaffold displays neurotrophic, neurogenic and anti-neuroinflammatory activities with high potential for development as a versatile CNS therapeutic. *Sci. Rep*, 7, 1492.
4. Karisetty BC et al (2017) Sex difference in mouse hypothalamic transcriptome profile in stress-induced depression model. *BiochemBiophys Res Comm* 486, 1122-1128.
5. Jhelum P et al (2017) Implications of epigenetic mechanisms and their targets in cerebral ischemia models. *Curr Neuropharmacol*, 15, 815-830.

Depression, anxiety and related disorders are debilitating mental illness, but unfortunately newer drugs with less side effects are still not in the offing. The reason for this is our failure to have better molecular insight into etiopathology of these psychiatric disorders. Recent research findings using diverse animal models have elegantly shown that dysregulation in epigenetic regulatory mechanisms in critical regions of neural circuitries results in altered gene/protein expression causing the development of depression and related mood disorder phenotype. Epigenetic mechanisms involving DNA and histones have been extensively studied in stress-induced animal models of mood disorders. However, there is another under-investigated layer of gene regulation mediated by non-coding RNA (ncRNAs), which has also been implicated in stress-induced neural and behavioural changes in models of depression which sometimes acts epigenetically. Our efforts in the last few years in this direction has led us to uncover a number of miRNAs, in addition to few piRNAs and lincRNAs, deregulated in the mouse cognitive circuitry in response to chronic stressors, which appear to be involved in depression-associated neural and neurogenic changes.

Using a chronic social defeat stress model of depression, our lab has identified hundreds of

miRNAs deregulated in the neurogenic region dentate gyrus (DG) of the mouse hippocampus. The most striking finding was the deregulation of most of the members of the mir-30 family, in neuroglial and behavioural changes associated with the affective disorder phenotype. Using high-throughput approaches such as miRarray, gene array, small RNA-seq and RNA-Seq, differentially regulated miRNA-mRNA networks were identified and a few of these were selected for further investigation. As the DG is the highly neurogenic region in the adult brain, adult mouse DG-derived differentiating and proliferating NSCs/NPCs were subjected to a miRNA array. To understand their modes of action, gene arrays were performed using samples from both the models/systems, where several dysregulated genes were identified. Using bioinformatics tools, predicted gene targets of these miRNAs were listed and compared with the dysregulated genes in our model systems to search for overlapping genes. To identify the direct targets of the miRNAs among the selected genes, luciferase reporter assays were performed. The studies have given us insights into the crucial role that a few selected miRNAs play through some of their gene targets in mediating the effects of chronic stress on neurogenesis in the social defeat mouse model of depression, anxiety, and related mood disorders.

Lekha Dinesh Kumar

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



From left to right: Anmol Kumar, Abdul Rawoof, Lekha Dinesh Kumar, Veena Gopinath, Sakshi Tiwari

RESEARCH INTERESTS:

- Deciphered important genes involved in the different grades and stages of colon cancer
- Mapped up/down regulated genes involved in EMT

Selected recent publications

1. Rasool R. et.al., 2017. AKT is indispensable for coordinating Par-4/JNK cross-talk in p21 downmodulation during ER stress. *Oncogenesis*. (In press).
2. Nayak D. et.al., 2017. Inhibition of Twist1-mediated invasion by Chk2 promotes premature senescence in p53-defective cancer cells. *Cell Death Differ*. 2017 Jul;24(7):1275-1287. doi: 10.1038/cdd.2017.70. Epub 2017 May 12.
3. Rasool R, et.al. 2016. Dual modulation of Ras-Mnk and PI3K-AKT-mTOR pathways: (2016). A Novel c-FLIP inhibitory mechanism of 3-AWA mediated translational attenuation through dephosphorylation of eIF4E. *Scientific Reports*.5;6:18800.

- Deciphered important genes involved in the different grades and stages of colon cancer
- Mapped up/down regulated genes involved in EMT

The initiation and progression of malignancy involves a series of molecular changes including various signals transduced during this process. *Wnt* signal transduction pathway is one of the major pathways deregulated in majority of cancers. Most sporadic colorectal cancers arise as a result of a mutation in the *Wnt*/ β -catenin pathway which in turn is critical towards colorectal cancer (CRC) progression. The key factor involved in this signaling pathway lies in the stabilization of β -catenin and its interaction with TCF transcription factors within the nucleus. In the cytoplasm the β -catenin is tightly regulated and the absence of *Wnt* factors results in its ubiquitination and degradation by the proteasome. Colon cancers frequently harbor genetic defects that inhibit the degradation of β -catenin which leads to nuclear β -catenin translocation and activation of target genes responsible for the *Wnt* activation. Constitutive transcription by the β -catenin/Tcf complex has been recognized as the key step for initiation of CRC. About 70% of all colorectal cancers show homozygous inactivation of the adenomatous polyposis coli (APC) tumor suppressor gene resulting in the activation of the *Wnt* signaling pathway and the constitutive transcription by the β -catenin/Tcf complex. Loss of APC function is present throughout the sequence of intestinal carcinogenesis (i.e., from benign adenomas to fully malignant colorectal cancer and metastasis).

Epithelial Mesenchymal transition (EMT) is often associated with embryonic development when major transitional events from blastula-morula-gastrula stages occur. This is a process with which embryonic cells loses its polarity and cell adhesion properties and acquire migratory and invasive

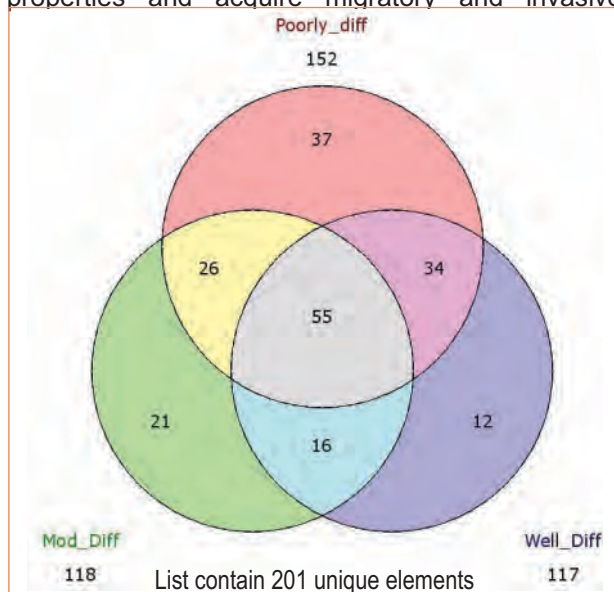


Fig.1: Venn diagram represents the differentially expressed genes in well differentiated, moderately differentiated and poorly differentiated human colon cancer samples which are > 1.5 fold up/down regulated compared to their respective adjacent normal sample

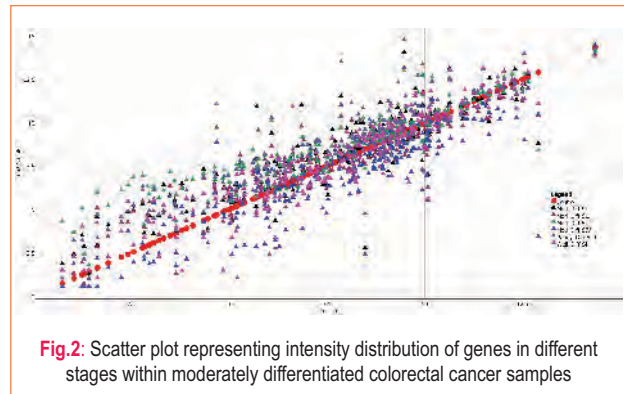


Fig.2: Scatter plot representing intensity distribution of genes in different stages within moderately differentiated colorectal cancer samples

properties of mesenchymal cells. During this process, many important genes responsible for cell division/multiplication would be down regulated and another set of genes responsible for migration of the cells will be activated associated with EMT process. The initiation and progression of cancers also involves such a phenomenon where the epithelial cells acquire more of mesenchymal properties for progression and invasion which leads into metastasis.

Cancers are thought to result from the accumulation of multiple genetic aberrations that transform cells, which allows for their abnormal growth, proliferation, and metastasis. Discovery of these aberrations and understanding how they contribute to the pathophysiology of cancer are necessary for improvements in diagnosis and therapy. More than any other model system, mice have revolutionized our ability to study gene function *in vivo* and understand the molecular mechanisms of cancer pathogenesis. As a model system, mice have several important advantages over other mammalian models having a small in size, rapid reproduction having large litters and they can be genetically manipulated.

The development of transgenic and gene targeting technologies facilitated the generation of genetically engineered mouse (GEM) models to study tumor

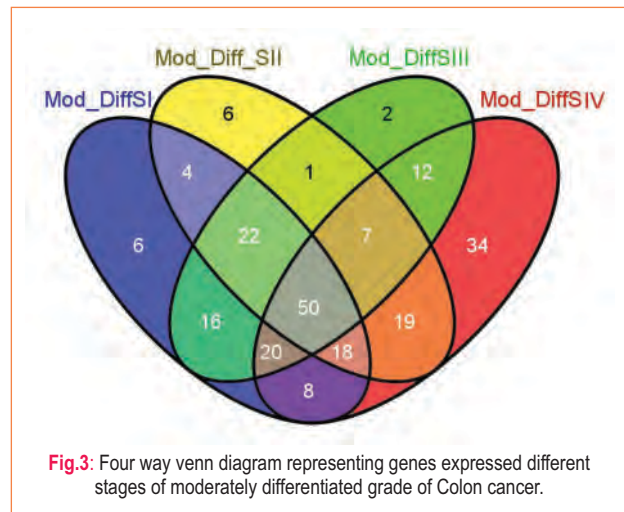


Fig.3: Four way venn diagram representing genes expressed different stages of moderately differentiated grade of Colon cancer.

Identified ~ 250 genes which are significantly deregulated in humans		
Deregulators	Human	Mouse
Ephb4	+++	+++
CITED-1	+++	+++
MMP2	+++	++
MMP9	+++	++
WNT-1	+++	++
Myc,stat1,3,Brca1,2	+++	++

Table.1: Highly upregulated genes found in different grades and stages of human cancer in common with Apc fl/fl mouse models of colon cancer
Expression of CITED-1 gene in stage III of moderately differentiated colorectal cancer samples

biology through the manipulation of the mouse germ line. The most common ways to generate mouse models of cancers are to activate oncogenes or inactivate tumor-suppressor genes (or both) *in vivo* through the use of transgenic and gene-targeting approaches, such as knockouts and knockins.

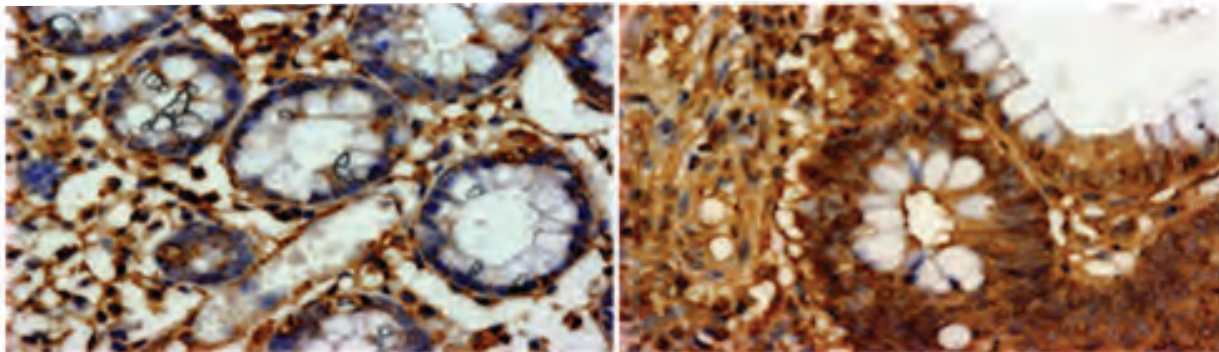
With an aim to decipher the wnt deregulators involved in the initiation and progression of colorectal cancers (EMT), microarray analysis was carried out in human colon cancer patients using nanostring technology. The human cancer genes were segregated based on their expression in well differentiated, moderately differentiated and poorly differentiated grades compared to their respective adjacent normals (Fig 1).

The genes expressed in the different stages of each grade which showed significant fold regulation (> 1.5 fold up/down regulated) compared to their adjacent normals were also classified. The scatter plot representing intensity distribution of these

genes are represented in Fig 2. Differentially expressed genes in different stages of moderately differentiated grade, stage I, II, III, IV has 144, 127, 130, 168 genes respectively compared to their respective adjacent normal samples. (Fig.3). These were then mapped into human chromosomes. These results were compared with the earlier results obtained from microarray analysis of murine colon cancer model (Apc fl/fl).

Epithelial mesenchymal transition being an important paradigm of cancer, it can be considered as a pathological process which contributes to cancer progression. Hence it is important to classify the genes responsible for progression of cancer from stage I to stage II which is often associated with EMT. After finding out the differentially expressed genes from different grades of colorectal cancer, we classified them based on the various progressive stages within each grade.

The various deregulated genes were classified as up and down regulated ones based on ≥ 1.5 fold and were placed on different signaling pathways associated with colon cancer. The status of these genes involved in various oncogenic pathways is shown below. The common genes of both mouse and human array experiments were selected for further studies. CITED-1 is a non-DNA binding transcription factor which has been reported to be deregulated in many cancers. We found (Fig 4) this to be up regulated 25 times in mice colon models and deregulated in human colon cancer samples using different platforms of microarray technique. In case of moderately differentiated colon cancer samples, cited-1 expression continuously increased from stage I (>1.5 fold change) to stage IV (>5). This was confirmed by immuno histochemistry as well as q-PCR in various grades and stages of human cancer samples.



Normal Tissue (40X)

Cancer Tissue (40X)

Fig.4: Expression of CITED-1 gene in stage III of moderately differentiated colorectal cancer samples

Satish Kumar

Functional Genomics using Transgenic and Knockout Mice and Molecular Approaches in Animal Breeding



From left to right (standing) : Ankita Chattopadhyay, Leena Kamalaskar, Manjusha Neerukonda, Sivapriya Pavuluri , Satish Kumar, Rajesh V Iyer, CB Tripura, Archana B Siva, Shalu Singh; (Sitting): Rohan Arora , M Manikandan, Arun Prakash Mishra, Alok Kumar, Rohit K Deshmukh

RESEARCH INTERESTS:

- Functional genomics using transgenic and gene knockout approaches in mice
- Understanding the genetics of livestock species

Selected recent publications

1. Singh VP, Katta S, Kumar S (2017). WD-repeat protein WDR13 is a novel transcriptional regulator of c-Jun and modulates intestinal homeostasis in mice. *BMC Cancer* 17(1):148.
2. Mitra S, Sameer Kumar GS, Tiwari V, Lakshmi BJ, Thakur SS, Kumar S (2016). Implication of Genetic Deletion of Wdr13 in Mice: Mild Anxiety, Better Performance in Spatial Memory Task, with Upregulation of Multiple Synaptic Proteins. *Front Mol Neurosci.* 9:73.
3. Whitelaw CB, Joshi A, Kumar S, Lillico SG, Proudfoot C (2016). Genetically engineering milk. *JDairy Res.* 83(1):3-11.

Molecular characterization of *Wdr13* knockout female mice uteri: A model for endometrial hyperplasia

Genotypic and phenotypic characterization of mutant mice exhibiting abnormalities in hair growth.

Wdr13, X-linked in both humans and mice, is an evolutionarily conserved gene expressed in several tissues including uteri. *Wdr13* knockout female mice develop endometrial hyperplasia indicating the functional significance of this gene in uterine tissue (Fig 1A, B). Endometrial hyperplasia is one of the leading causes of cancer in women, where most of the genes that promote cell cycle inhibition are known to be downregulated. The *Wdr13* knockout mice uteri showed downregulated expression of cell cycle inhibitor genes, cyclin G2 and p21 (Fig 1C). Further, *in-vitro* experiments have demonstrated that the over expression of *Wdr13* led to an increased expression of cyclin G2 and p21 mRNA indicating that *WDR13* affects the expression of these genes. *Wdr13* mutant mice also showed an increase in the weight of abdominal and ovarian fat pad combined with elevated levels of estradiol; all of which are known risk factors for endometrial hyperplasia (Fig 1D, E). Interestingly, we also found that *WDR13* acts as a co-repressor for estrogen receptor-alpha (*ERα*) which might be affecting the expression of target genes containing the estrogen responsive elements in mutant mice. The current study, thus demonstrated the importance of *WDR13* in mouse

uterine tissue. The mimicking of endometrial hyperplasia condition along with the associated metabolic disorders, make this mouse strain a potential disease model to study endometrial hyperplasia in humans.

In another study, we have identified a mutant mouse that loses pelage hair completely by post-natal day 21, regrows hair until day 40, and thereafter has a permanent "Sparse hair" phenotype that is more prominent on the dorsal side of the abdomen (Fig 2A). Mating experiments show that the underlying mutation in these mice is autosomal recessive with 100% penetrance and expressivity. Other co-occurring abnormalities along with the hairless phenotype include: (i) gum inflammation and altered length of incisors, (ii) splenomegaly, (iii) significant neonatal lethality in pups born to homozygous parents on post-natal day 1 (P1), and (iv) prominent blood vessels on the ear when compared to that of the wildtypes (Fig 2A). Post-mortem analyses of P1 pups revealed empty stomachs in all of them (n=5). Analyses of milk from mutant dams demonstrated a significant reduction in the total triglycerides and cholesterol levels as compared to those in wildtypes (Fig 2B). Profiling of serum for biochemical and liver

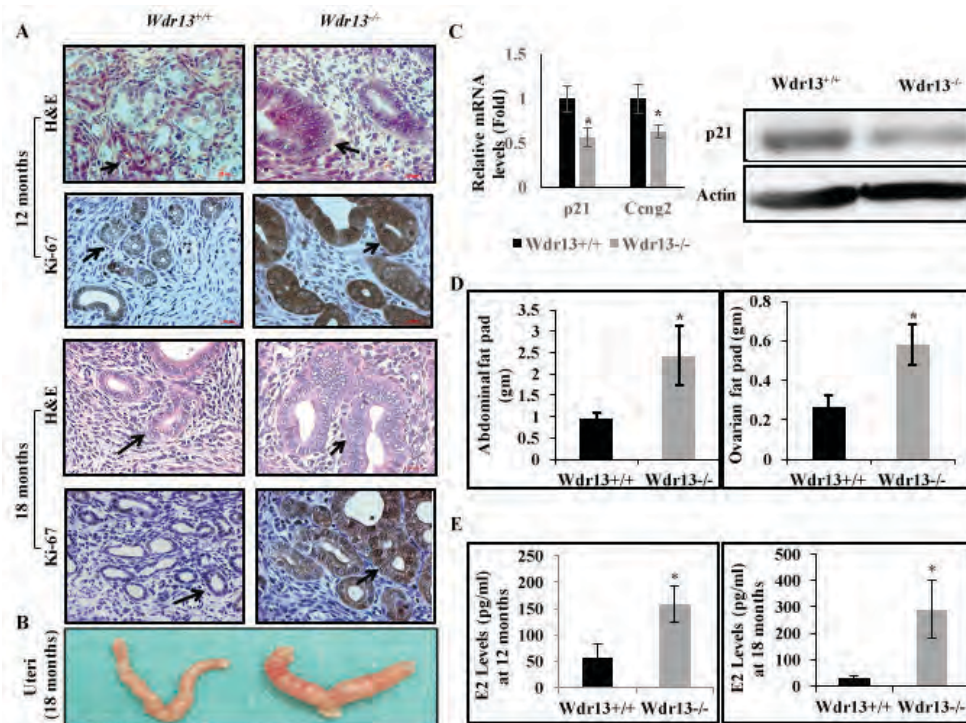


Fig.1: Morphological and gene level analyses of *Wdr13*^{+/+} and *Wdr13*^{-/-} female mice uteri. A) H&E and Ki-67 staining of uteri cross sections belonging to *Wdr13*^{+/+} and *Wdr13*^{-/-} mice of age 12 and 18 months (n=5). B) Gross anatomical structure of *Wdr13*^{+/+} and *Wdr13*^{-/-} mice uteri at 18 months of age. C) Expression levels of p21 and cyclinG2 in *Wdr13*^{+/+} and *Wdr13*^{-/-} mice (n=5). D) Analysis of abdominal fat pad (n=7) and ovarian fat pad (n=7) in *Wdr13*^{+/+} and *Wdr13*^{-/-} mice. E) Analysis of estradiol levels in *Wdr13*^{+/+} and *Wdr13*^{-/-} mice at 12 and 18 months of age (n=5).

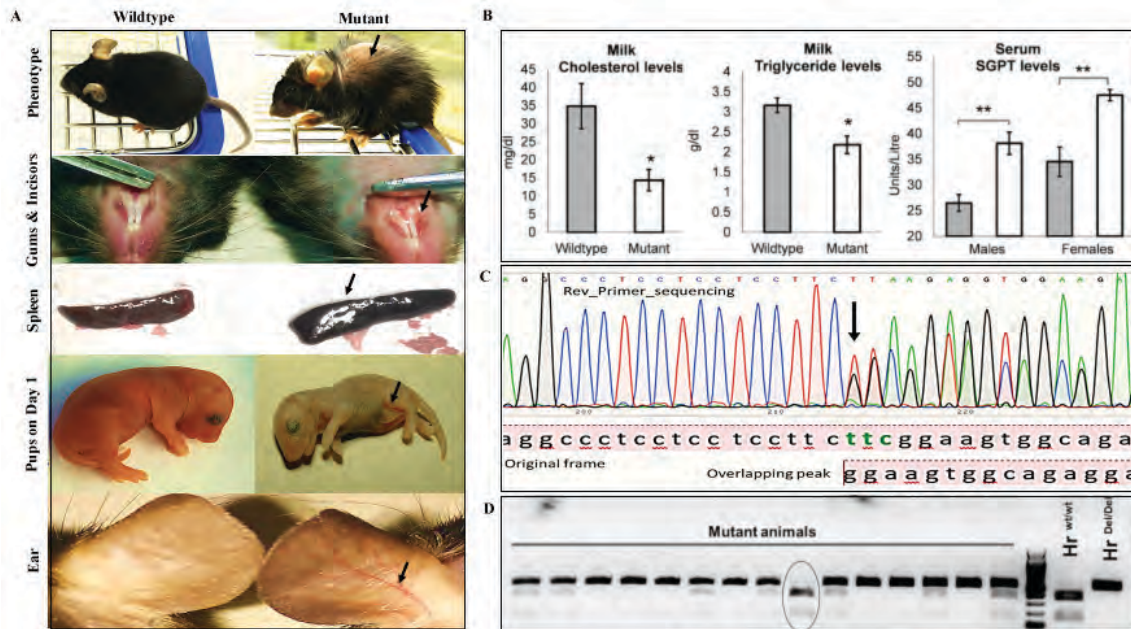


Fig.2: Phenotypic and genotypic characterization of mutant C57BL/6J mice. A) Morphological characteristics of mutant mice exhibiting sparse hair phenotype, inflammation of gums with altered length of incisors, splenomegaly, neonatal lethality in day1 pups and prominent blood vessels in the earas compared to those of wildtype. B) Analyses of levels of triglycerides and cholesterol in milk, and serum SGPT in wildtype and mutant mice. C) Sanger sequencing of *Hr* exon-6 with reverse primers showing the trinucleotide deletion-TTC. D) PCR-RFLP genotyping for *Hr*- exon6 trinucleotide deletion using *Mbo*-II restriction enzyme shows that a mutant animal does not carry this particular deletion.

function parameters such as SGPT (Fig 2B) indicates a metabolic syndrome in the mutant pups born to and suckled by mutant females. Sequencing of *Hr* gene- a commonly mutated gene in hair loss conditions, revealed a novel heterozygous trinucleotide deletion in Exon 6 (Fig 2C). This deletion is in-frame and removes a Glutamic acid residue at position 534 of the protein. Multiple sequence alignment of *hr* orthologues from mouse, human, rhesus macaque, rat, pig, and cow showed that the corresponding Glutamic acid residue is conserved. Surprisingly, in a larger panel of mutant animals, this deletion did not segregate with the observed phenotype in our study (Fig 2D).

In the area of livestock genetics, an ongoing collaborative project (with Roslin Institute, UK and

BAIF Research Foundation, Pune), focuses on understanding the genetic basis of resistance/ predisposition to tuberculosis and brucellosis in Indian buffalo. An extensive molecular epidemiological survey was carried out in the breeding tracts of Jaffarabadi buffalo to identify Tuberculosis and Brucellosis positive animals. Genome of 36 animals (6 each of Murrah, Jaffarabadi, Banni, Pandharpuri, Surti and Bhadawari breeds) have been sequenced using NGS platform. Efforts are ongoing to generate a reference genome of Jaffarabadi buffalo by combining NGS data with PacBio sequencing. A fine scale atlas of gene expression in three breeds of domestic riverine water buffalo (Mediterranean, Pandharpuri and Bhadawari) has been generated through RNA-sequencing.

Mukesh Lodha

Mechanism of Epigenetic Cellular Memory Inheritance in Plants



From left to right : Akanksha Garhwal, Supriya Sarma, Preethi Jampala, Asha Ganpatrao Mane, Mukesh Lodha, Sai Deep, Aditya Undru

RESEARCH INTERESTS:

- Mechanism of inheritance of epigenetic cellular memory
- Chromatin mediated regulation of developmental genes

We have a strong interest in elucidating mechanisms involved in the 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.

Selected recent publications

1. Lodha M, Marco CF, and Timmermans MCP (2013). Direct Recruitment of Polycomb Repressive Complex2 by the *Arabidopsis* ASYMMETRIC LEAVES Complex. *Genes and Development*. 27:596-601.
2. Husbands AY, Benkovics AH, Nogueira FT, Lodha M and Timmermans MC (2015). The ASYMMETRIC LEAVES Complex Employs Multiple Modes of Regulation to Affect Adaxial-Abaxial Patterning and Leaf Complexity. *Plant Cell*. 27(12):3321-35.
3. Lodha M, Schulz-Raffelt M and 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(1):172-6.
4. Schulz-Raffelt M, Lodha M and Schroda M. Heat shock factor 1 is a key regulator of the stress response in *Chlamydomonas*. *Plant J*. 52(2):286-95 (2007).
5. Lodha M and Schroda M (2005). Analysis of chromatin structure in the control regions of the *chlamydomonas* HSP70A and RBCS2 genes. *Plant Mol Biol*. 59(3):501-13.

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 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.

Histone post translational modifications, DNA methylation and non-coding RNA can potentially transmit memory during cell division. As a first step in understanding the mechanism of epigenetic inheritance, we are trying to identify histone tail residues which are important in epigenetic inheritance of memory of cold in *Arabidopsis*. We are using transgenic plants expressing c-terminally tagged and mutants in N-terminal tail amino acid residues. These plants will be tested in a vernalization (mitotically stable memory of cold) paradigm to test their ability to gain and retain epigenetic memory. We will test the proteins which are involved in turnover of the known histone post translational modifications on the identified histone tail residue for their roles in inheritance of epigenetic memory. 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 SHOOT MERISTEMLESS (STM), an important shoot meristem stem cell regulator and a determining factor in leaf complexity, as a tool to understand epigenetic regulation in plants. In simple leaved

species such as *Arabidopsis thaliana* STM is expressed in the shoot apical meristem and is down regulated 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 down regulation 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 a compound leaf expression pattern when introduced into *Arabidopsis thaliana*. These experiments establish 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 down regulated in the leaf primordia in simple leaf species like *Arabidopsis thaliana* by transcriptional repressors, Polycomb complexes. It is tempting to hypothesize that binding of PRC2/transcriptional repressors to cis regulatory elements 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 are trying to identify and characterize the cis regulatory elements in the simple leaf species *Arabidopsis thaliana* and compound leaf species *Cardamine hirsuta* which allows differential expression of STM in these two species as a model of leaf complexity in plants, and to establish role of chromatin in differential regulation of STM.

M M Idris

Bio-mechanisms of Regeneration and Degeneration



Mohammed Idris, Mir Quoseena, Naga Sowmya

RESEARCH INTERESTS:

- Bio-mechanism of Regeneration
- Bio-mechanism of Degeneration

Our group works on understanding the bio-mechanisms involved in the regeneration of organs and the degeneration of brain structures in humans, 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 is likely to lead to a better understanding of the underlying mechanisms.

Selected recent publications

1. Babu NS, Murthy CL, Kakara S, Sharma R, Swamy CV, Idris MM (2016) MPTP induced Parkinson's disease in zebrafish. *Proteomics* 16(9): 1407- 1420.
2. Babu NS, Krishnan S, VenakataSubbaiah GP, Gurava Reddy VG, Swamy CV, Idris MM (2016) Quantitative proteomic analysis of normal and degenerated human intervertebral disc. *Spine J.* 16(8): 989- 1000.
3. 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 Regen.* 24(3): 551-559.
4. Purushothaman S, Saxena S, Meghah V, Swamy CVB, Ortega-Martinez O, Dupont S, Idris MM (2015) Transcriptomic and proteomic analyses of *Amphiura filiformis* arm tissue-undergoing regeneration. *J Proteomics* 112:113- 124.
5. Purushothaman S, Saxena S, Meghah V, Meena Lakshmi MG, Singh SK, Swamy CVB, Idris MM (2015) Proteomic and gene expression analysis of zebrafish brain undergoing continuous light/dark stress. *J Sleep Res.* 24(4): 458 - 465.

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 regeneration of the zebrafish caudal fin. 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. The roles of epigenetic regulatory mechanisms, such as histone H3 and H4 lysine acetylation and methylation during zebrafish caudal fin regeneration were also studied using CHIP and RT-PCR assays. 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.

We are also involved in understanding the mechanism of degeneration in human intervertebral disc (Degenerative disc disease) and neurodegenerative disorders such as Spinocerebellar ataxia (SCA) and Parkinson's disease (PD) in the zebrafish model. A total of 759 and 692 proteins respectively, were identified as differentially regulated in the Annulus fibrosus and Nucleus pulposus tissues of the human intervertebral disc based on proteomics studies. Neuro-degeneration, the chronic breakdown of neuronal structures, is mostly associated with expansion of CAG triplet repeats leading to aggregation and polymerization of proteins. Our laboratory aims to understand the association of expanded triplet repeats (CAG) in the ataxin gene with neurodegeneration based on a transgene approach in zebrafish. We have also identified the role of chronic unpredictable stress and chemicals such as MPTP involved in inducing degeneration of the zebrafish nervous system.

M V Jagannadham

Studies on Outer Membrane Vesicles of Bacteria



M V Jagannadham, P Gayathri, Deepika

RESEARCH INTERESTS:

- Structural and functional studies of outer membrane vesicles of bacteria
- Chemical derivatization of proteins and peptides for facilitated analysis by mass spectrometry
- Detecting Post translational modifications of proteins using Mass spectrometry

Our group identified The N-terminal amino acid residues for the tryptic peptides generated from *in gel* digested proteins after acetylating the peptides and detecting b1 ions in the mass spectra.

The methods developed helps in improving the *de novo* sequencing efficiency of peptides using mass spectrometry.

Selected recent publications

1. Bhanuramanand K, Sankararao K, Jagannadham MV, Swamy Ch. V B, Dholpe, V.M. and Rao, N M (2017) Combinatorial labelling method for improving peptide fragmentation in Mass spectrometry. *J. Amer. Soc. Mass spectrum*. 28(6): 1216-1226.
2. Chattopadhyay MK and Jagannadham MV (2015) Vesicles-mediated resistance to antibiotics in bacteria. *Front. Microbiol.* 6:758. Doi: 10.3389/fmicb.2015.00758.
3. Kulkarni HM, Nagaraj R and Jagannadham MV (2015) Protective role of *E. coli* outer membrane vesicles against antibiotics. *Microbiol. Res.* 181: 1-7.
4. Fulsundar S, Kulkarni HM, Jagannadham MV, Nair R, Sravani Keerthi, Sant P, Pardesi K, Bellare J and Chopade BA (2015) Molecular characterization of outer membrane vesicles released from *Acinetobacter radioresistens* and their potential roles in pathogenesis. *Microbial Pathogenesis* 83-84: 12-22.
5. Kulkarni HM and Jagannadham M V (2014) Biogenesis and multifaceted roles of outer membrane vesicles from Gram-negative bacteria. *Microbiology* 160: 2109-2121.

Role of OMVs from *Acenitobacter baumannii* in antibiotic resistance

Acenitobacter baumannii, an opportunistic human pathogen, is responsible for several diseases like pneumonia, meningitis and a wide range of other infections. It is also typically involved in nosocomial infections. Outer membrane vesicles (OMVs) released from the bacteria play an important role in the bacterial physiology including pathogenesis. They also contribute to antibiotic-resistance of bacteria in various ways. The present investigation was undertaken to look into the molecular basis of vesicle-mediated antibiotic-resistance in bacteria. OMVs were obtained from a clinical, multidrug resistant, nosocomial strain of *A. baumannii*, isolated by Ms Bina Agarwal under the supervision of Prof. G. Ahmed at the University of Guwahati. The strain was originally obtained from a tertiary care hospital in Guwahati, Assam. The OMVs were prepared from the bacteria, the protein bands from the OMVs were cut and subjected to *in gel* digestion using trypsin. The peptides were extracted using standard protocols and subjected to LC coupled ESI MS/MS studies. The proteins were identified by SEQUEST. A total of 320 proteins were identified in OMVs produced by the antibiotic-sensitive strain, and 560 proteins were identified from the vesicles produced by the multi-drug resistance (MDR) strain. An overlap of 15 proteins was observed, 545 proteins were exclusively present in MDR OMVs and 305 proteins were specific from the sensitive strain OMVs. A comparative proteomics analysis of the OMVs from the *A. baumannii* sensitive and MDR strain revealed the increased content of carbapenemase and a host of other related antibiotic-inactivating enzymes in the OMVs of the *A. baumannii* MDR strain. Growth studies on the *A. baumannii* and MDR strain in the presence of polymixin B and OMVs prepared from the respective strains revealed that the OMVs protected the bacteria against the antibiotic. These studies reveal that OMVs may play an important role in antibiotic resistance/persistence.

Identification of proteins from species of unknown genome sequences (collaboration with Dr R Srinivas, CSIR-IICT, Hyderabad)

The proteins from the *Acenitobacter radioresistance*, whose genome sequence is not known, were chosen to analyse using MS/MS spectra. The proteins were identified using a database prepared from the

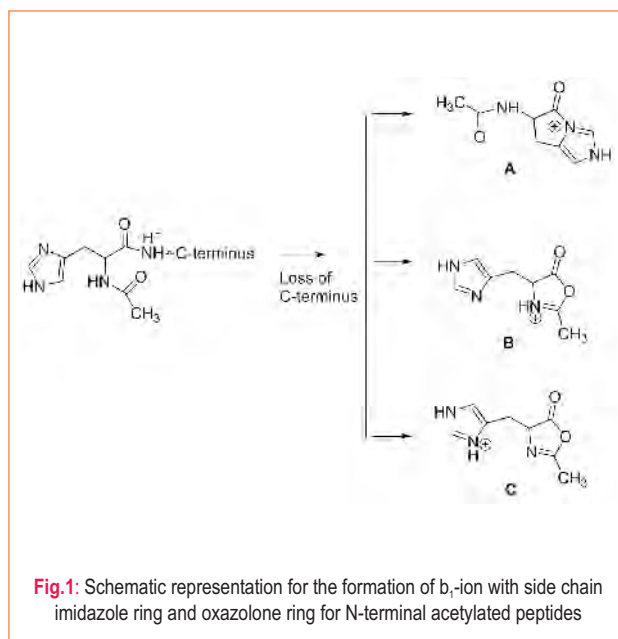


Fig.1: Schematic representation for the formation of b₁-ion with side chain imidazole ring and oxazolone ring for N-terminal acetylated peptides

genome sequences of the other species of *Acenitobacter*. After extracting the total proteins of the organism, equal amounts of the extract were loaded into two different wells in 12% SDS-Polyacrylamide gel. The protein bands obtained in both the lanes were excised and subjected to *in gel* trypsin digestion. The digested bands in one lane were identified by LC coupled ESI-MS/MS analysis. The digested bands from the other lane were acetylated and subsequently identified by LC coupled ESI-MS/MS analysis. The peptides containing N-terminal histidine were analysed in detail to understand the mechanism for the formation of b₁ ion. N-terminal histidine containing peptides were shown to form b₁ ion, by cyclization with the side chain imidazole (Fig 1A).

The detection of post translational modifications (PTMs) of proteins is an important comprehensive research subject. Among all possible downfalls that may lead to misidentifications, the chemical stability of modified peptides is scarcely questioned. Global proteomic studies devoted to protein acetylation are becoming attractive. Thus, we were concerned about the acetylation of histidine and its intrinsic stability of b₁-ion of the Oxazolone ring and/or with side chain imidazole bicyclic product. Because of the formation of an oxazolone structure may occur when an amino moiety has gone through an acetylation reaction and when it is present in the vicinity of the side chain imidazole moiety. Here, we probed the possibility of a ring formation between oxazolone and a bicyclic compound with the side chain imidazole. Tryptic

peptides containing N-terminal histidine was explored in a standard proteomic workflow. We have validated the formation of the Oxazolone ring through a low energy calculation of density functional theory (DFT) studies. This computational method suggested that the conformer (C) in which the protonation has occurred at the imidazole ring is more stable than the other two conformers namely A and B. Among all these conformers, A is unstable than C by ~24 kcal/mole and the other conformer B, is intermediate between both the A and C conformers. The results obtained in the present study showed that the histidine had undergone acetylation and that the N-acetylated group also may participate in ring formation. This was the case for the N-acetylated peptide. In this case, the oxazolone ring with N-acetyl group may also form rather only with the side chain imidazole group, indicating that N-terminal N-acetylated histidine forming oxazolone ring may be stable and will be one of the products in the proteomics workflow. The N-terminal N-acetyl histidine oxazolone ring obtained, has low energy and stable structure in proteomic samples. We also report the shift of MS to differentiate between peptides containing acetylated histidine from the unacetylated moiety by showing a clear mass shift. Further, studies are in progress to characterize the immonium ions and b-ions after acetylation of tryptic peptides. These studies will help in improving the *de novo* sequencing efficiency of the peptides, in addition to validate the sequence of peptides.

Detecting multiple phosphorylations in peptides using MS

A wide variety of post-translational modifications such as oxidation, phosphorylation, glycosylation, methylation and acetylation play critical roles in cellular functions. Detection of PTMs in proteins is important to understand their crucial roles in cellular functions. Identifying each modification requires special attention in mass spectral acquisition and analysis. Here, we report a mass spectral method for the detection of multiple phosphorylations by analyzing the fragments. Synthetic peptides were used to identify these modifications by Matrix-assisted laser desorption/ionization (MALDI) TOF/TOF. Peptides with serine, threonine and tyrosine were used with mono- to tetra-phosphorylation sites in different combinations to get insights into their fragmentation and identify the location of these sites. The γ -ions series were observed without the loss of phosphate groups and were thus very useful in determining the localization and sequence of the phosphate residues. Acetylation of the peptides was found to be useful in detecting the b1 ion and helped in identifying the N-terminus. When a mixture of the phosphorylated peptides (from mouse protein sequences) was analyzed by LC-MS/MS on a VelosOrbitrap Mass Spectrometer and the data were subjected to analysis by Sequest using the mouse database, the peptides were identified along with the parent proteins. A comparison of MALDI TOF/TOF spectra with ESI MS/MS helped in eliminating falsely discovered peptides using the database search.

Rakesh K Mishra

Genome Organization and Epigenetic Regulation



M Arif, F Athar, Akshay Kavvaru, Ashish Bihani, V Chakrapani, Nikhil Hajimis, Runa Hamid, Alshymaa Hassan, Shagufta Khan, Gopal Kushwah, Shreyashi Mukherjee, M Nisha, Indira Paddibhatla, Rashmi U Pathak, K Phanindhar, Titus Ponarantnam, Parna Saha, Ravina Saini, Salam Herojeet Singh, Divya Tej Sowpati, A Srinivasan, Avinash Srivastava, Ishani Srivastava, Surabhi Srivastava, Rahul Sureka, Shreekanth Verma, Mukesh P Yadav, Rakesh K Mishra

RESEARCH INTERESTS:

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

Selected recent publications

1. Akshay Kumar A, Saketh S, DivyaTej S and Rakesh K. Mishra (2017) MSDB: A Comprehensive Database of Simple Sequence Repeats. *Genome Biol Evol* 9(6):1797-1802.
2. Krishnan J, Fathima A, Swaroopa Rani T and Rakesh K. Mishra (2017) Simple sequence repeats showing 'length preference' have regulatory functions in humans. *Gene* 628:156-161.
3. Gopal Kushwah and Rakesh K. Mishra (2017). Ultraconserved sequences associated with HoxD cluster have strong repression activity. *Genome Biol Evol* 9 (8): 2049-2054.
4. Debaditya De, Satish K, Jae-Min Lim, Rashmi U Pathak and Rakesh K. Mishra (2017). O-GlcNAcylation of Boundary Element Associated Factor (BEAF 32) in *Drosophila melanogaster* correlates with active histone marks at the promoters of its target genes. *Nucleus* doi: 10.1080/19491034.2017.1367887.
5. DivyaTej S, Surabhi S, Jyotsna D and Rakesh K. Mishra (2017). C-State: An interactive web app for simultaneous multi-gene visualization and comparative epigenetic pattern search. *BMC Bioinformatics* : doi.org/10.1101/163634.

We developed a Comprehensive Database of Simple Sequence Repeats (MSDB) and demonstrated that SSRs that are enriched in complex genomes have diverse functions

Earlier we identified conserved non-coding sequences associated with the Hox clusters that, as we now show for the first time, have a repressive function

We demonstrate that post transcriptional modifications of BEAF, a boundary element associated factor, determine its functional specificity

Packaging of genomic DNA has regulatory consequences on the expression of genes during development. This regulation is based on chromatin structure in which the 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:

MSDB: A Comprehensive Database of Simple Sequence Repeats

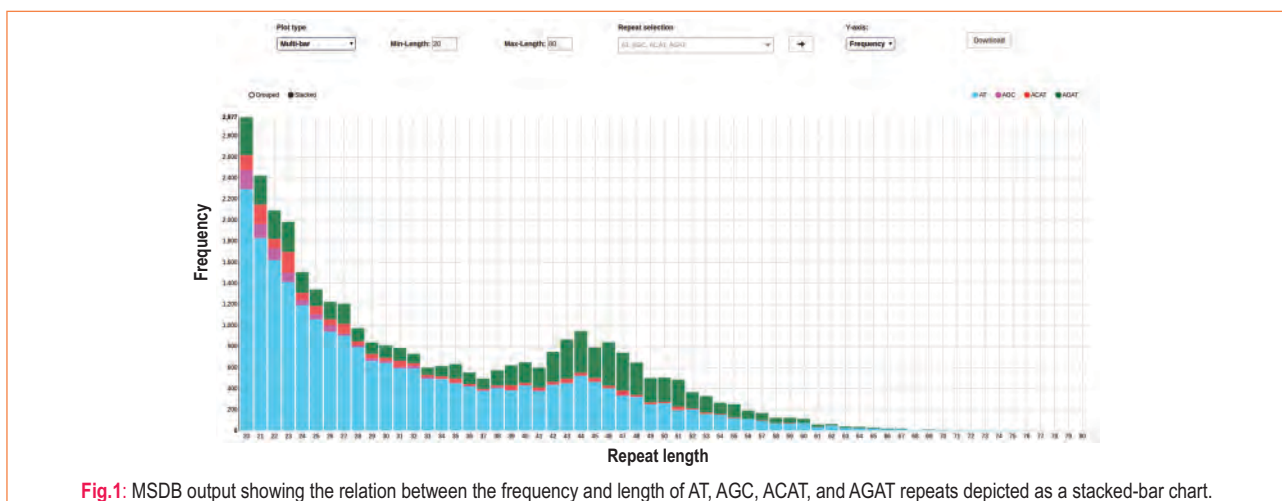
Microsatellites, also known as Simple Sequence Repeats (SSRs), are short tandem repeats of 1-6 nt motifs present in all genomes, particularly eukaryotes. Besides their usefulness as genome markers, SSRs have been shown to perform important regulatory functions, and variations in their length at coding regions are linked to several disorders in humans. Microsatellites show a taxon-specific enrichment in eukaryotic genomes, and some may be functional. Despite being an important class of regulatory elements, there is no

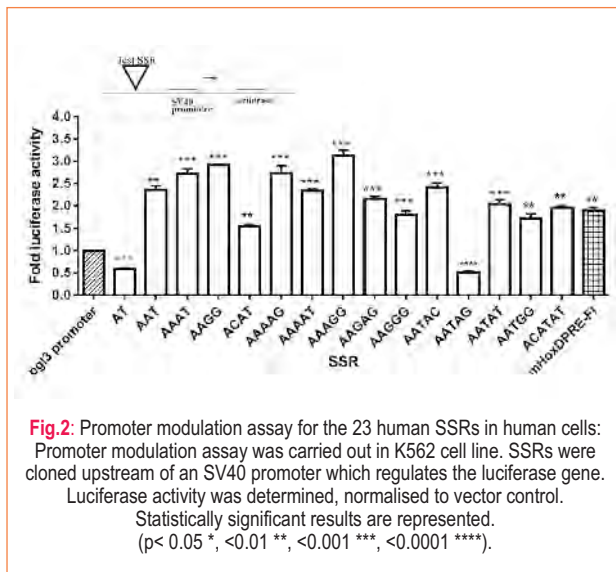
comprehensive database of microsatellites across various organisms. The existing databases are either specific to a certain taxon (e.g., FishMicroSat and Plant microsatellite database), or have information for a limited number of species. In addition, many of the existing databases were released several years ago, and have not been updated with the currently available sequence information. More importantly, the data is generally provided in a static tabular format, which makes data exploration and observation of global trends cumbersome. Finally, no existing database provides a direct means to compare the microsatellite data of multiple species, making evolutionary analysis of these elements laborious and challenging.

Using the available genome sequence information from NCBI, we created a database of SSRs from almost all available species that is thorough, up-to-date, and enables users to easily query and explore microsatellite data of several species simultaneously. MSDB (Microsatellite Database) is a collection of >650 million SSRs from 6,893 species including Bacteria, Archaea, Fungi, Plants, and Animals. This database is by far the most exhaustive resource to access and analyze SSR data of multiple species. In addition to exploring data in a customizable tabular format, users can view and compare the data of multiple species simultaneously using our interactive plotting system. MSDB is developed using the Django framework and MySQL. It is freely available at <http://tdb.ccmb.res.in/msdb>.

Simple sequence repeats showing 'length preference' have regulatory functions in humans

Analysis of genomes across various taxa show that despite the instability associated with longer stretches of repeats, a few SSRs with specific longer repeat lengths are enriched in the genomes indicating



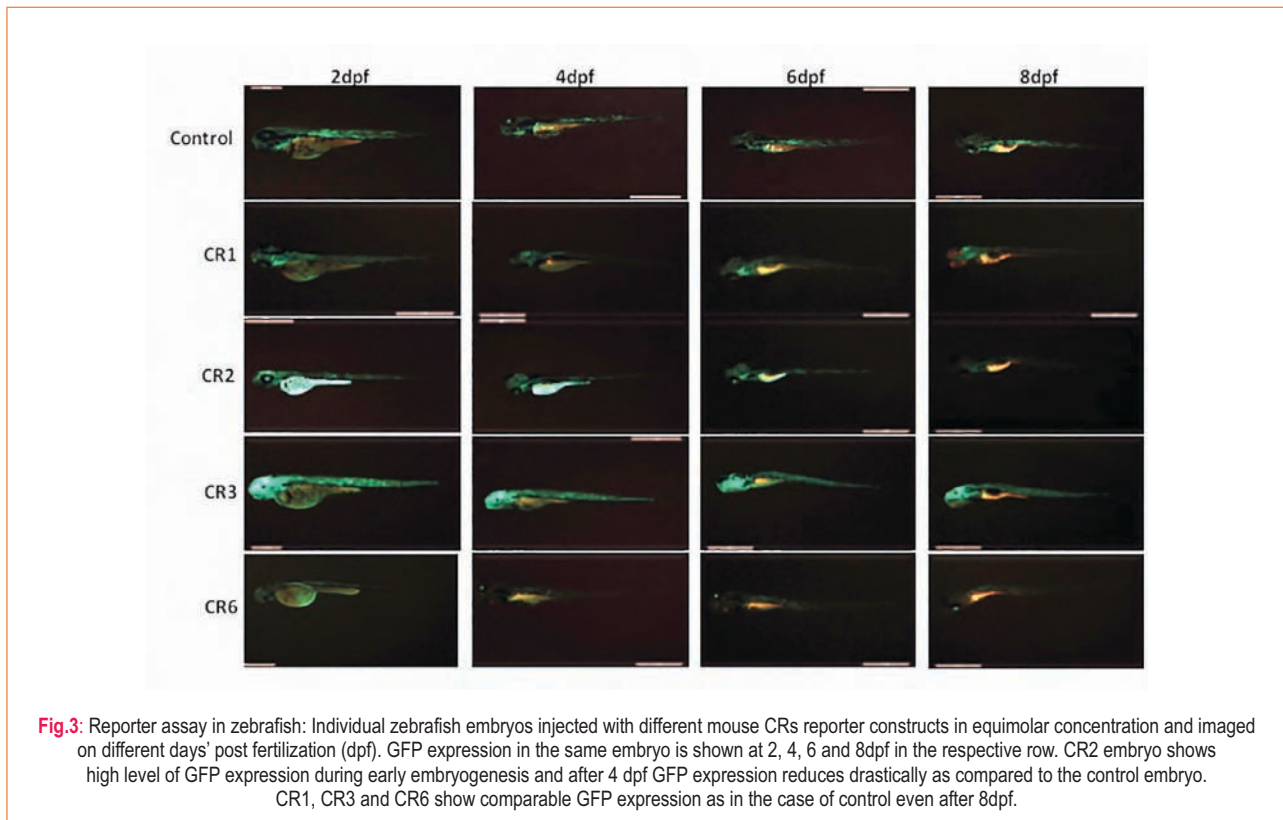


a positive selection. This conserved feature of length dependent enrichment hints at not only sequence but also length dependent functionality for SSRs. We selected 23 SSRs of the human genome that show specific repeat length dependent enrichment and analysed their cis-regulatory potential using promoter modulation, boundary and barrier assays. We find that the 23 SSR sequences, which are mostly intergenic and intronic, possess distinct cis-regulatory potential. They modulate minimal promoter activity in transient luciferase assays and are capable of functioning as enhancer-blockers and

barrier elements. The results of our functional assays propose cis-gene regulatory roles for these specific length enriched SSRs and opens avenues for further investigations.

Ultra-conserved sequences associated with the HoxD cluster have strong repression activity

An increase in the complexity of organisms during evolution strongly correlates with the increase in the non-coding DNA content of their genomes. Although a gradual increase in the proportion of repetitive DNA elements along with increasing complexity is known, most of the non-coding components of the genome remain uncharacterized. A non-repetitive but highly conserved non-coding component of the genome in vertebrates, called ultra-conserved DNA sequences, constitutes up to 5% of the human genome. The functions of most of the ultra-conserved DNA elements are not well understood. One such ultra-conserved stretch of DNA has been identified upstream of the HoxD cluster in vertebrates. We analyzed the function of these elements in different cell lines and zebrafish. Our results suggest that these ultra-conserved sequences work as repressor elements. This is the first report which reveals the repressor function of ultra-conserved sequences and implicates their role in the regulation of developmental genes.



O-GlcNAcylation of Boundary Element Associated Factor (BEAF 32) in *Drosophila melanogaster* correlates with active histone marks at the promoters of its target genes:

BEAF 32 is a sequence specific DNA binding protein involved in functioning of chromatin domain boundaries in *Drosophila*. Several studies also show it to be involved in transcriptional regulation of a large number of genes, many of which are annotated to have cell cycle, development and differentiation related function. Since post-translational modifications (PTMs) of proteins add to their functional capacity, we investigated the PTMs on BEAF 32. The protein is known to be phosphorylated and O-GlcNAcylated. We mapped the O-GlcNAc site at T91 of BEAF 32 and showed that it is linked to the deposition of active histone (H3K4me3) marks at the transcription start site (TSS) of associated genes. Its role as a boundary associated factor, however, does not depend on this modification. Our study shows that by virtue of O-GlcNAcylation, BEAF 32 is linked to epigenetic mechanisms that activate a subset of associated genes.

C-State: An interactive web app for simultaneous multi-gene visualization and comparative epigenetic pattern search

Comparative epigenomic analysis across multiple genes presents a bottleneck for bench biologists working with NGS data. Despite the development of standardized peak analysis algorithms, the identification of novel epigenetic patterns and their visualization across gene subsets remains a challenge. We developed a fast and interactive web app, C-State (Chromatin-State), to query and plot chromatin landscapes across multiple loci and cell types. C-State has an interactive, JavaScript- based graphical user interface and runs locally in modern web browsers that are pre-installed on all computers, thus eliminating the need for cumbersome data transfer, pre-processing and prior programming knowledge. C-State is unique in its ability to extract and analyze multi-gene epigenetic information. It allows for powerful GUI-based pattern searching and visualization. In conclusion, C-State is a standalone application for epigenetic and gene expression analysis, providing an easy solution for experimental

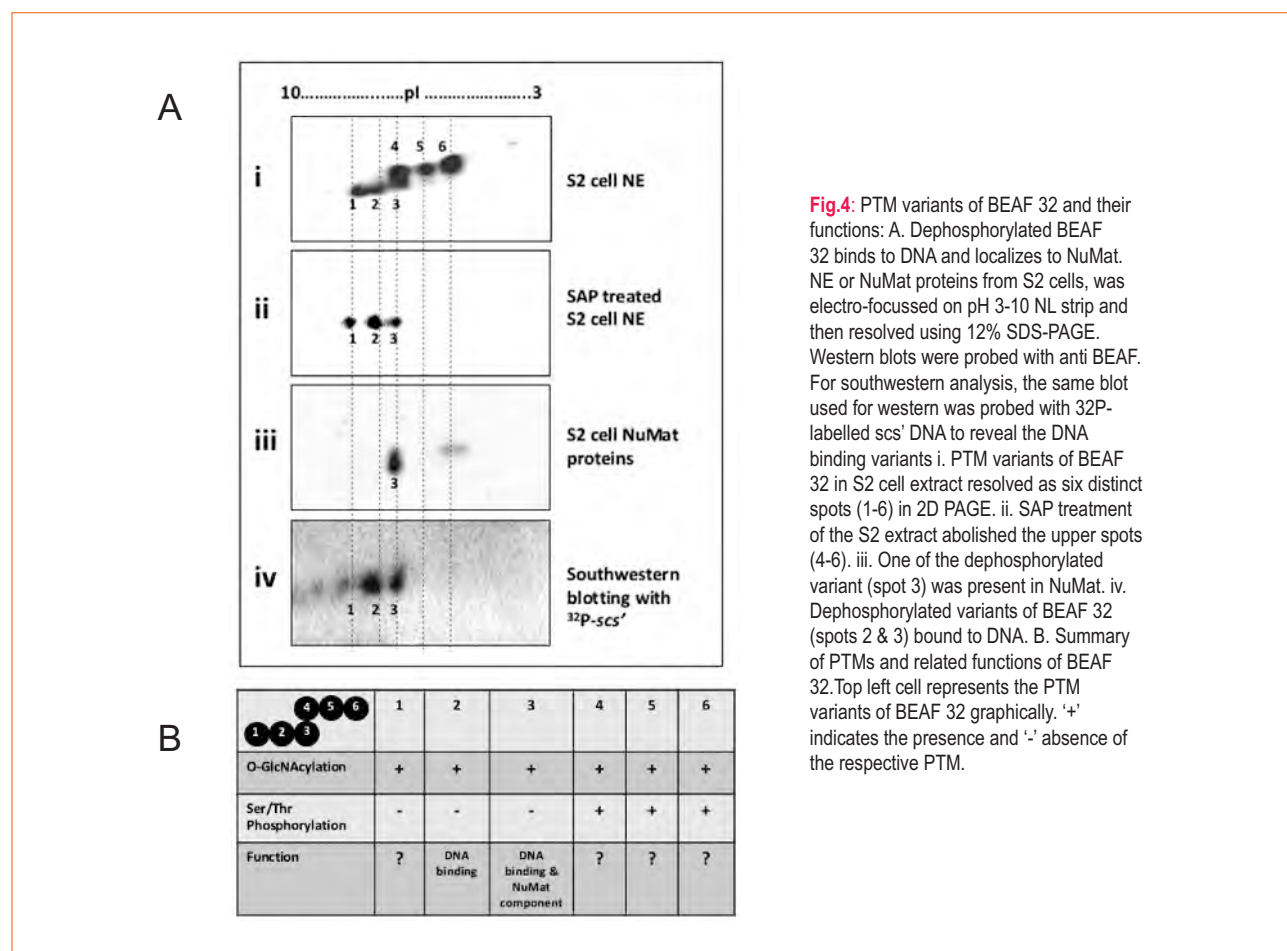


Fig.4: PTM variants of BEAF 32 and their functions: A. Dephosphorylated BEAF 32 binds to DNA and localizes to NuMat. NE or NuMat proteins from S2 cells, was electro-focussed on pH 3-10 NL strip and then resolved using 12% SDS-PAGE. Western blots were probed with anti BEAF. For southwestern analysis, the same blot used for western was probed with ³²P-labelled scs' DNA to reveal the DNA binding variants i. PTM variants of BEAF 32 in S2 cell extract resolved as six distinct spots (1-6) in 2D PAGE. ii. SAP treatment of the S2 extract abolished the upper spots (4-6). iii. One of the dephosphorylated variant (spot 3) was present in NuMat. iv. Dephosphorylated variants of BEAF 32 (spots 2 & 3) bound to DNA. B. Summary of PTMs and related functions of BEAF 32. Top left cell represents the PTM variants of BEAF 32 graphically. '+' indicates the presence and '-' absence of the respective PTM.

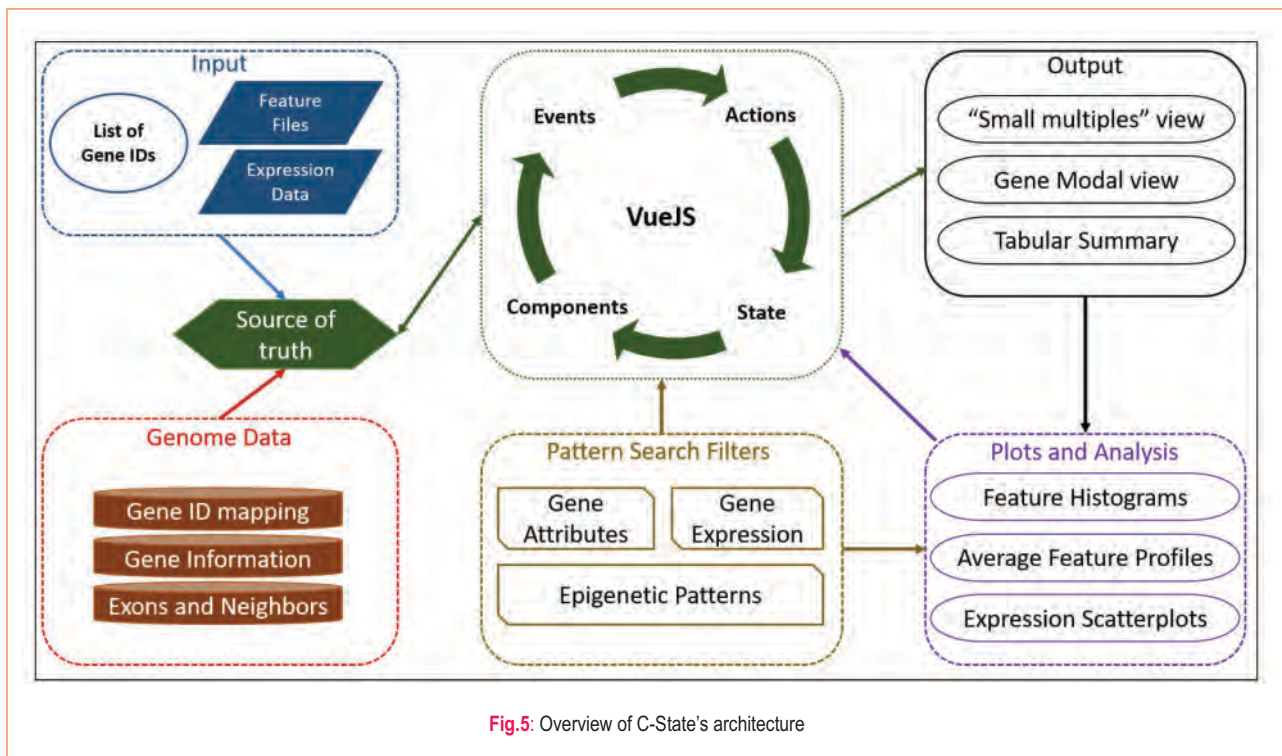


Fig.5: Overview of C-State's architecture

biologists to investigate epigenetic patterns without needing to know how to handle or parse big data. In addition to running locally on the user's system, it can also be hosted centrally on an internal network to which multiple users can connect for visualizing and sharing their data. C-State's searchable filtering and display modules are extensible and can handle data from any organism for a large number of genes and a flexible amount of intergenic regions. This is very useful for

researchers to analyze novel or publicly available datasets in order to formulate new hypothesis for experimental testing, without investing in complicated programming or bioinformatics help. C-State also allows the incorporation of user-generated data with published datasets for rapid comparison and analysis and facilitates easy documentation of salient information via capture of high quality, publication ready images. The tool is available at <http://www.ccmb.res.in/rakeshmishra/c-state/>

P Chandra Shekar

Early Embryonic Development in Mouse



From left to right : Vishnu Vijay, Usha Kabilan, Hanuman Kale, Debabrata Jana, P Chandra Shekar

RESEARCH INTERESTS:

- Early embryonic development in mouse
- Understanding cell fate choice based on transcription factor modulation in pluripotent state
- Genetic disease modeling using pluripotent stem cells.

We are trying to understand how cell fate decisions are made in the early stages of embryogenesis and during stem cells differentiation based on the expression levels of key transcription factors. We use functional analysis and biochemical approaches to perturb transcription factor networks to understand underlying mechanisms.

Selected recent publications

1. Miguel F, Chandra Shekar P, Yen-Sin A, Fujiwara Y, Stuart H. Orkin, Jianlong W (2011) Zfp281 Functions as a Transcriptional Repressor for Pluripotency of Mouse Embryonic Stem Cells. *Stem Cells* 29:1705–1716.
2. Chandra Shekar P, Adnan Naim P, ParthaSarathi D and Satish Kumar (2011) Argonaute-2-null embryonic stem cells are retarded in self-renewal and differentiation. *J. Biosci.* 36: 649-657.
3. P. Chandra Shekar, Sandeep Goel, S. Deepa Selvi Rani, D. Partha Sarathi, Jomini Liza Alex, Shashi Singh, and Satish Kumar; (2006) κ -Casein-deficient mice fail to lactate. *Proc. Natl. Acad. Sci. USA* 103:8000-8005.
4. Ravishankar K V, Chandra Shekar P, Sreedhara SA, Dinesh LalithaAnand M R, Saiprasad GVS; (2004) Diverse genetic bases of Indian polyembryonic and monoembryonic mango (*Mangifera indica* L.) cultivars. *Current Science* 87:870-871.

We are studying an extended regulatory network comprising signaling 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 core pluripotency networks

We have earlier shown that unlike previously thought, Oct4 and Nanog do not activate each other. In certain conditions no correlation or an inverse correlation exists between expression of Nanog and Oct 4. ChIPseq analysis of previous published data has identified an interesting relationship between regulation of Nanog and Oct4. Nanog binding on Oct4 promoter does not change with concentration, however Oct4 binding to the nanog promoter and its activation are dependent on the concentration of Oct4. Our current experiments are focused on understanding how changes in concentration of Oct4 affect the expression of Nanog leading to a particular cell fate choice. We are also interested in understanding how changes in levels of Oct4 or Nanog are perceived by cells and how cell fate decision are made based on the concentration of either Oct4 or Nanog. In this direction we have developed a ligand binding destabilization system using an Auxin inducible degradation system for Nanog. We have established a titratable controlled expression of Nanog in ES cells to carry out biochemistry experiments.

Nanog auto repression loop

The NANOG mediated auto repression loop ensures that the expression level of NANOG does not exceed the normal self-renewal limits. The ZFP281 and

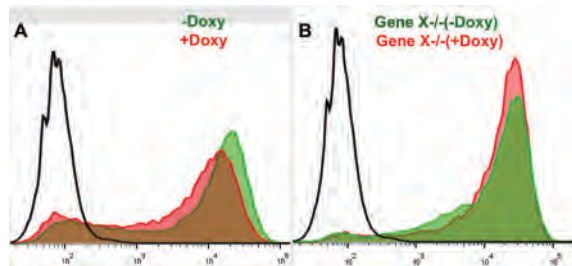


Fig.1: (A) Repression of Nanog GFP reporter upon induction of Nanog protein overexpression by doxycycline. (B) Failure of repression of Nanog GFP in the Nanog downstream target gene-knock out cell line by doxycycline induction

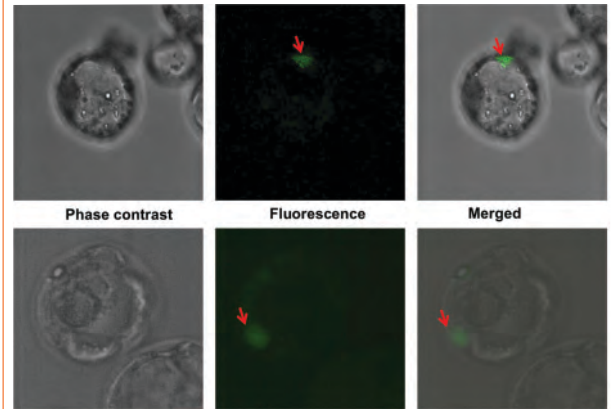


Fig.2: GFP labelled ES cells treated in new culture condition were injected into morula and contribution of green cells (ES) to trophoblast is marked with an arrow.

NuRD complexes are essential for NANOG auto repression. Data from our lab suggests that ZFP281 and NuRD are essential but not sufficient for auto repression of NANOG. We have identified a downstream target of Nanog which gets transcriptionally activated in just 1 hour post Nanog induction. Further our experiments suggest that the downstream target is essential for auto repression of Nanog (Fig.1). Small molecule inhibition of the same downstream target also leads to loss of Nanog auto feedback repression further confirming our observations.

Differentiation of pluripotent ES cell to trophoblast lineage

ES cells can differentiate into all the 3 germ layers of the body -ectoderm, mesoderm and endoderm. However ES cells cannot give rise to all extra embryonic layers particularly the trophoblast implying that they are not totipotent like a zygote. In our lab we have carried out a small-scale screen of small molecules to identify culture conditions which can differentiate ES cells into a trophoblast lineage. In this new culture condition a small population of ES cells differentiate to the trophoblast lineage. Specific changes in the expression of core pluripotency factors like Oct4 and Nanog promote or prevent differentiation of ES cells into a trophoblast lineage under this culture condition. The ES cells not only differentiate to the trophoblast lineage in vitro but they also contribute to the trophoblast of developing blastocysts when the treated ES cells are injected into the morula (Fig. 2).

Since the new culture condition extends the differentiation potential of ES cells to trophoblasts, it has implications in developmental biology and stem cell biology studies.

Veena K Parnaik

Nuclear Organization and Lamin Biology



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

RESEARCH INTERESTS:

- Effects of laminopathic mutants on differentiation and development
- Mechanism of protein degradation induced by laminopathic mutants

Selected recent publications

1. Verma, AD and Parnaik VK (2017) Heart-specific expression of laminopathic mutations in transgenic zebrafish. *Cell Biol Int* 41:809-819.
2. Verma, A D and Parnaik VK (2015) Identification of tissue-specific regulatory region in zebrafish laminA. *Gene* 567:73-80.
3. Chaturvedi P, Khanna R and Parnaik VK (2012) Ubiquitin ligase RNF123 mediates degradation of heterochromatin protein 1 α and β in laminA/C knock-down cells. *PLoS ONE* 7: e47558.
4. Chaturvedi P and Parnaik VK (2010) LaminA rod mutants target heterochromatin protein 1 and for proteasomal degradation by activation of F-box protein, FBXW10. *PLoS ONE* 5: e10620.
5. Parnaik VK (2008) Role of nuclear lamins in nuclear organization, cellular signaling and inherited diseases. *Int Rev of Cell Mol Biol* 266: 157-206.

We have characterised transgenic zebrafish models of heart-specific laminopathies

The lamins are major components of a filamentous network of proteins termed the nuclear lamina that lies beneath the inner nuclear membrane in a metazoan cell. Lamins are important for the organization of nuclear functions as well as chromatin organization, and have also been proposed to play key roles in cell differentiation pathways. Two major kinds of lamins are present in higher eukaryotes. The B-type lamins are expressed in all somatic cell types whereas the expression of A-type lamins is restricted to differentiated cells of most lineages. Mutations in the human laminA gene (*LMNA*) have been associated with at least 15 debilitating inherited diseases, collectively termed laminopathies, that affect specific tissues such as skeletal muscle, cardiac muscle, adipose tissue and bone, and also cause premature ageing syndromes. A few laminopathies have been studied using the mouse as a model organism.

The current research in my group is directed towards investigating the effects of laminopathic mutations on cellular functions and embryonic development. The zebrafish has emerged as a useful model system for the study of vertebrate development, especially due to the ease of visualization of GFP-tagged genes during embryogenesis in transgenic fish. Zebrafish has four genes that code for lamins A, B1, B2 and LIII. Zebrafish laminA gene shows 63% identity and 76% similarity with the human lamin A gene. Based on its conserved gene structure and sequence similarity, zebrafish lamin A can be considered to be an orthologue of mammalian lamin A. As zebrafish is a well-established model for the study of cardiac development and disease, we have investigated the effects of heart-specific laminA mutations in transgenic zebrafish. We have developed transgenic lines of zebrafish expressing conserved laminA mutations that cause cardiac dysfunction in humans. Expression of zlamina mutations Q291P and M368K in the heart was driven by the zebrafish cardiac troponin T2 promoter. Homozygous embryos displayed nuclear abnormalities in ~20% of cardiomyocyte nuclei. Expression analysis showed the upregulation of genes involved in heart regeneration in transgenic mutant embryos and a cell proliferation marker was increased in heart tissue. At the physiological level, there was deviation of up to 20% from normal heart rate in transgenic embryos expressing mutant lamins. Adult homozygous zebrafish were fertile and did not show signs of early mortality. Our results suggest that transgenic

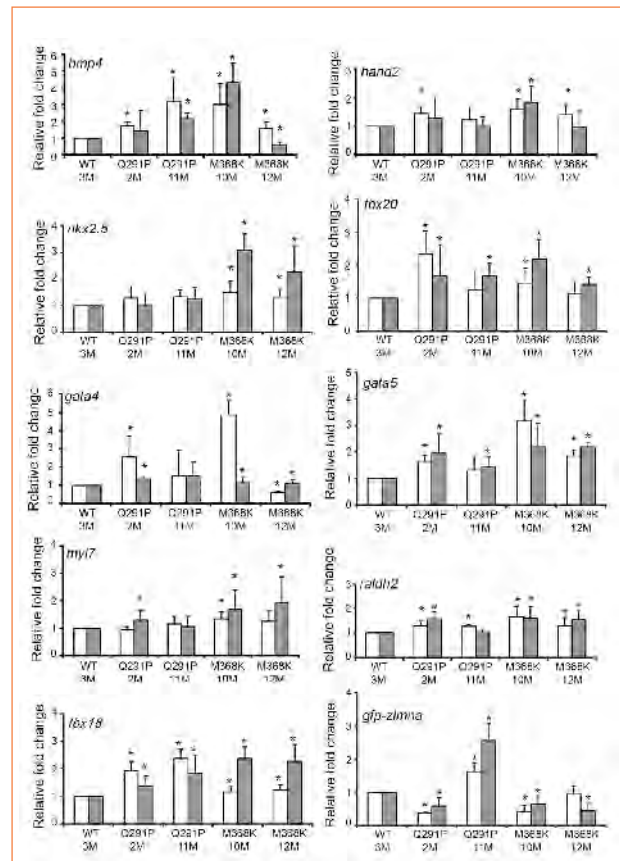


Fig.1: Quantitative RT-PCR analysis of cardiac developmental and regeneration genes in transgenic embryos at 3.5 days post fertilization (open bars) and 5.5 days post fertilization (solid grey bars); * $p \leq 0.05$

zebrafish models of heart-specific laminopathies show cardiac regeneration and moderate deviations in heart rate during embryonic development.

An emerging concept in lamin pathogenesis is that laminopathic mutations alter interactions of lamins with key regulatory factors and chromatin bound proteins. We have earlier shown that laminopathic mutations can induce ubiquitin-mediated proteasomal degradation of specific chromatin-binding proteins such as heterochromatin protein 1 (HP1) isoforms and the DNA damage sensor, ATR (Ataxia-telangiectasia-mutated-and-Rad3-related) kinase by activation of specific E3 ubiquitin ligase components, namely, the monomeric RING ligase RNF123, the HECT ligase HECW2 and the substrate adaptor for a multimeric RING ligase FBXW10. Our current studies on the identification of additional substrates for these ligases indicate that lamin-binding proteins are important targets for RNF123 and HECW2. Our findings imply that ubiquitination and degradation of lamin-binding proteins may contribute to disease-causing mechanisms in laminopathies by depletion of key nuclear proteins.

Anant B Patel

¹³C Nuclear Magnetic Resonance Investigations of Neurotransmitter Energetics in Neurological Disorders



From left to right : Pooja, Kamal, Arun, Anant, Sampath, Narayan, Rajan, Dipak, Pravin, Naveleen, Madhuri

RESEARCH INTERESTS:

- Development of ¹³C NMR methods to study Brain Energy metabolism
- Excitatory and Inhibitory neurotransmission in neurodegenerative disorders
- Neuronal and astroglial metabolic activity in psychiatric disorders
- Development of MRI contrast materials

We have shown that the energetics of Glutamatergic and GABAergic neuro-transmission is distinct across brain.

In a contradiction of Astrocyte-to-Neuron Lactate Shuttle hypothesis we have shown direct uptake and phosphorylation of glucose in neurons.

Impairment of neurometabolism at preclinical stage provides a novel insight for development of biomarkers in diagnosis of Alzheimer's disease.

Reduction in neuronal activity in prefrontal cortex plays an essential role in the pathophysiology of depression.

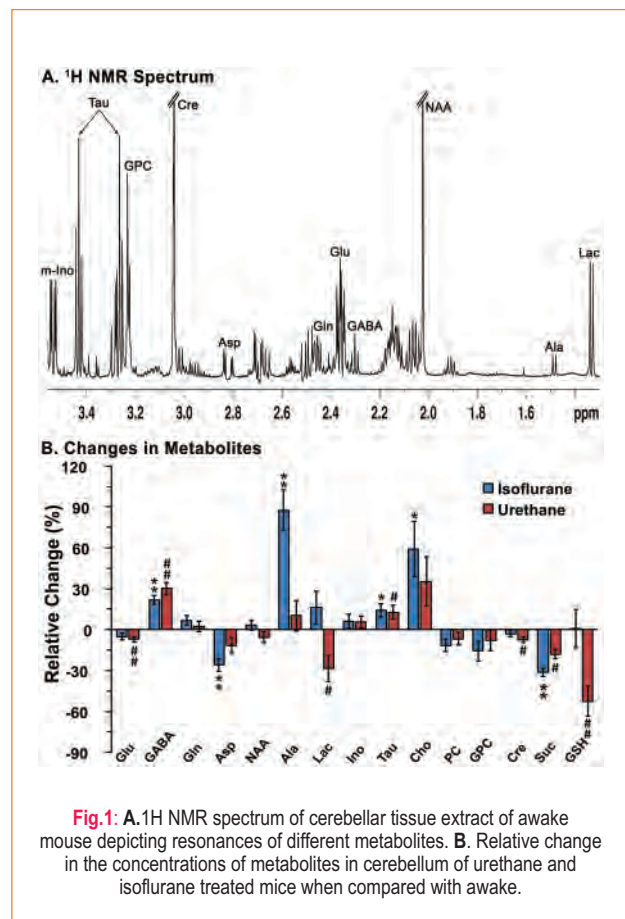
Selected recent publications

1. Patel AB, Tiwari V, Veeraiah P, Saba K (2017) Increased astroglial activity and reduced neuronal function across brain in A β PP-PS1 mouse model of Alzheimer's disease. *J Cereb Blood Flow Metab* doi:10.1177/0271678X17709463.
2. Bagga P, Behar KL, Mason GF, De Feyter HM, Rothman DL, Patel AB (2014) Characterization of Cerebral Glutamine Uptake from Blood in the Mouse Brain: Implications for Metabolic Modeling of ¹³C NMR Data. *J Cereb Blood Flow Metab* 34:1666-1672.
3. 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. *Biol Psychiatry* 76:231-238.
4. 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. *Proc Natl Acad Sci USA* 111:5385-5390.
5. Bagga P, Chugani AN, Varadarajan KS, Patel AB (2013) *In Vivo* NMR Studies of Regional Cerebral Energetics in MPTP Model of Parkinson's Disease: Recovery of Cerebral Metabolism with Acute Levodopa Treatment. *J Neurochem* 127:365-77.

Although the human brain represents only ~2% of the body weight, it accounts for ~20% of total oxygen utilization of the whole body, indicating the overwhelming energy demands of the brain. Metabolic and neurophysiological research has experimentally related brain energy consumption, in the form of glucose oxidation, to the brain work supporting neuronal firing. Glutamate and -aminobutyric (GABA) acid are the major excitatory and inhibitory neurotransmitters respectively, in the matured mammalian central nervous system. *In vivo* ^{13}C NMR spectroscopy studies have shown that the rates of neurotransmitter cycle and neuronal glucose oxidation are stoichiometrically coupled indicating neuronal energetics is supported by oxidative glucose metabolism. Our research interest lies in the development and application of ^{13}C NMR methods to investigate neuro transmitter energetics in different neurological conditions using ^{13}C NMR spectroscopy together with an infusion of ^{13}C labeled substrates (glucose, acetate, β -hydroxybutyrate). Metabolism of $[1,6-^{13}\text{C}_2]$ glucose in the glutamatergic and GABAergic neurons labels Glu_{C_4} and GABA_{C_2} during the first turn of the TCA cycle. The trafficking of Glu_{C_4} and GABA_{C_2} into astrocytes incorporates label into Gln_{C_4} . Due to the exclusive presence of monocarboxylate transporter on astrocytes, $[2-^{13}\text{C}]$ acetate is selectively transported and metabolized therein, and labels Gln_{C_4} . The labelling of the neurotransmitters Glu_{C_4} and GABA_{C_2} occurred via neurotransmitter cycling between astroglia and neurons. Hence, the ^{13}C turnover of amino acids provides quantitative information about neuronal & astroglial metabolic activity and neurotransmitter cycling in the brain.

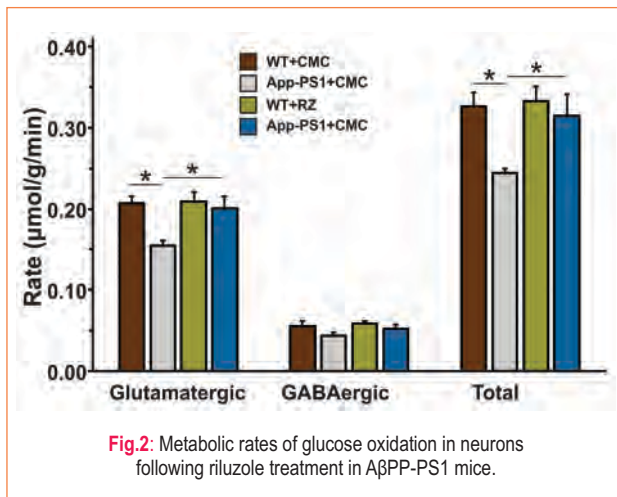
Anesthetics play significant role during surgical interventions. Different anesthetics have been used to investigate neurometabolism in rodents but their impact on the homeostasis of different neurometabolites, and brain function is not well understood. We have evaluated the impact of two anesthetics, urethane (injectable) and isoflurane (volatile), on the homeostasis of neurometabolites in the brain. For this, male C57BL6/J mice were anesthetized using isoflurane (1.2-1.5%) and urethane (1.5 g/kg, i.p.) for 45 min, and euthanized using focused beam microwave irradiation. The level of neurometabolites were measured in brain tissue extract *ex vivo* in ^1H NMR spectrum (Fig. 1A).

Exposure with isoflurane led to a significant reduction in the levels of aspartate and succinate, and an increase in alanine level in all brain regions studied. The level of glutamate was decreased in the cerebral



cortex, striatum and prefrontal cortex in mice administered with urethane, while it was lowered in the striatum, thalamus-hypothalamus and cerebellum under isoflurane. GABA level was reduced in the cerebral cortex under isoflurane but it was increased in the cerebellum and olfactory bulb under urethane and isoflurane (Fig. 1B). Additionally, taurine levels decreased in the cerebral cortex and striatum in urethane treated mice, while they were elevated in the cerebellum and olfactory bulb in mice anesthetized with urethane as well as isoflurane. These data suggest isoflurane and urethane perturb neurometabolites homeostasis differentially across the brain.

We are studying the pathophysiology of Alzheimer's disease (AD) using a transgenic mouse model. Our earlier studies have indicated that neuronal activity is decreased while astroglial function is enhanced in AD. Although different approaches have been used to combat AD, there has been very limited success. We have evaluated the efficacy of riluzole, an FDA approved drug for Amyotrophic lateral sclerosis, for improvement of neurometabolism in AD mice. Cerebral metabolism was followed using ^{13}C NMR spectroscopy in tissue extracts following an infusion of $[1,6-^{13}\text{C}_2]$ glucose. The concentrations of ^{13}C labeled



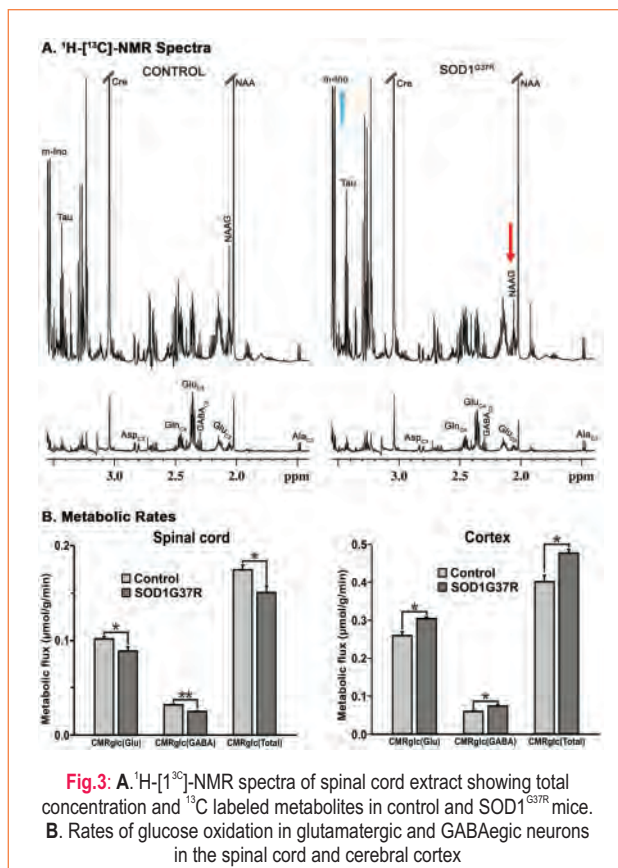
amino acids were found to be significantly lower in AβPP-PS1 as compared with age matched controls suggesting glucose hypometabolism by glutamatergic neurons in the cerebral cortex. The administration of riluzole in AβPP-PS1 mice increased ¹³C labeling of brain amino suggesting improved cerebral metabolic rate of glucose oxidation to the control level (Fig. 2). As rate of neuronal glucose oxidation is stoichiometrically coupled with neurotransmitter cycling, our finding of increased neuronal glucose oxidation with riluzole suggest improved neurotransmission in AD mice. These data suggest that riluzole has the potential to manage cognitive function in Alzheimer's disease.

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by selective degeneration of upper and lower motor neurons leading to progressive muscle weakness and eventual death as a result of respiratory failure. We are studying the pathophysiology of ALS using SOD1^{G37R} mice. These mice exhibit reduced paw grip strength and rigid paralysis as compared to wild type controls.

The analysis of neurometabolites indicated decreased level of N-acetyl aspartate and increased myo-inositol level in SOD1^{G37R}, which suggest neuronal death and neuroinflammation in the spinal cord and brain stem of ALS mice (Fig. 3A). SOD1^{G37R} mice exhibit decreased labeling of glutamate-C4, GABA-C2 and glutamine-C4 from [1,6-¹³C₂]glucose when compared with controls, suggesting impaired cerebral metabolic rates of glucose oxidation by glutamatergic and GABAergic neurons in the spinal cord under ALS condition (Fig 3B). In contrast, the metabolic activity of excitatory (glutamatergic) and inhibitory (GABAergic) neurons was enhanced in the cerebral cortex and striatum.

These data suggest distinct pathology in the spinal cord and brain in ALS condition.

We are studying the pathophysiology of depression using social defeat and (SD) and chronic unpredictable mild stress (CUMS) models of depression. Earlier, we have shown compromised excitatory and inhibitory neuronal activity in the prefrontal cortex under depression. We have evaluated the flux through the pentose phosphate pathway (PPP) in the social defeat model of depression. The ¹³C labeling of different isotopomers of amino acids labeled from [1,2-¹³C₂]glucose were measured using ¹³C-[¹H]-NMR spectroscopy. The labelling of [4-¹³C]glutamate was lower in SD mice when compared with controls. The PPP flux was significantly decreased in the cerebral cortex as well as in sub-cortical region in SD mice. The reduction in PPP flux was more in the sub-cortical region as compared with that in the cerebral cortex. PPP generates NADPH, which acts as a cofactor for glutathione reductase, an enzyme involved to maintain glutathione in the reduced form. The finding of reduced PPP flux in cerebral cortex and subcortical region suggests a compromised antioxidant defense system of neural cells in SD mice that might be responsible for reduced neuronal function in depression.



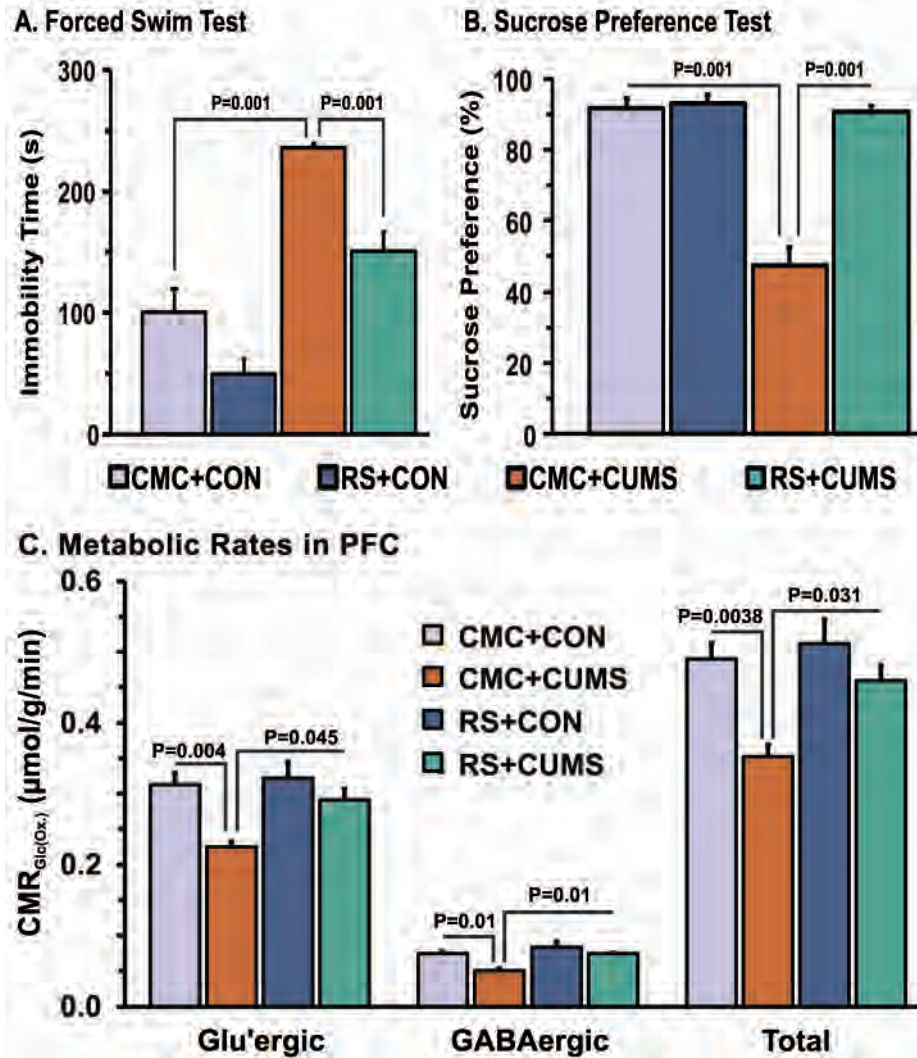


Fig.4: Effects of Rasa Sindoor supplementation on behavior phenotype: **A.** Forced Swim test and **B.** Sucrose preference, and **C.** Neuronal Metabolic Rates in PFC of CUMS mice.

We are using SD and CUMS models to evaluate efficacy of different intervention in depression. Recently, we have shown that intervention of NMDA receptor antagonist in SD/CUMS mice restored the behavioral phenotype and neuronal activity to the control level. We have explored the impact of pre-supplementation of Rasa Sindoor, a formulation used in the Indian traditional medicine, on behavior and neurometabolic activity in CUMS model of depression. Prior supplementation of RS in CUMS mice decreased the immobility time (Fig. 4A) and increased sucrose preference (Fig. 4B) when

compared to vehicle administered CUMS. The ¹³C labeling of prefrontal cortex amino acids was increased significantly in RS supplemented CUMS mice when compared to vehicle treated, and was not significantly different from vehicle treated controls.

These data suggest that prior supplementation with RS maintained the neuronal glucose oxidation by excitatory (glutamatergic) and inhibitory (GABAergic) neurons (Fig. 4C), and neurotransmission in CUMS mice. Therefore, RS supplementation will be beneficial to combat chronic mild stress in humans.

R Nagaraj

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



From left to right : Ankeeta Guru, Basil Mathew, R Nagaraj

RESEARCH INTERESTS:

- How the host-defense antimicrobial peptides, defensins exert their activity and to explore routes of developing them as future therapeutic agents

Selected recent publications

1. Pachahara SK, Subbalakshmi C, Nagaraj R (2017). Formation of Nanostructures by Peptides. *Curr Protein Pept Sci.*; 18:920-938.
2. Mathew B, Nagaraj R (2017). Variations in the interaction of human defensins with *Escherichia coli*: Possible implications in bacterial killing. *PLoS One* (In Press).

Our study on the activity of defensin analogs on biofilms provides significant insights into the structural requirements for disrupting pre-formed biofilms by human defensin analogs. Further, D-enantiomers of defensins could become potential therapeutic candidates for treating biofilm-associated infections.

Interaction of defensin analogs with biofilms

Biofilms are complex sessile communities of microorganisms. More than 80% of the medically relevant microbial infections are associated with biofilms. Therefore, there is an urgent need to develop new therapeutic agents to overcome the challenges associated with biofilm-associated infections. Recent studies indicate that cationic host-defense peptides are promising agents against biofilms. Defensins are one of the largest class of host-defense peptides present in humans. It has been reported that human β -defensin 3 (HBD3) exhibits potent anti-biofilm activity, even against antibiotic resistant bacterial biofilms. We have generated a large number of human defensins HBD 1-4, HD5 and 6 analogs with antibacterial activity against planktonic forms of *Escherichia coli* and *Staphylococcus aureus*. This library of analogs can be classified into four different groups: 1) linear analogs; 2) linear fatty acylated analogs; 3) disulfide containing analogs; 4) chimeric analogs of HBD-1 and theta defensin. Their effect on biofilms have not been explored. In this study, we have examined the effect of these defensin analogs on pre-established biofilms of *E. coli* and *S. aureus*.

Linear and linear fatty acylated defensin analogs did not show any effect on biofilms. Chimeric analogs hBTD-1 and its D-enantiomer [D]hBTD-1 eradicate *S. aureus* biofilm efficiently with a minimum biofilm eradication concentration (MBEC) of 150 μ M and 200 μ M, respectively. Although analogs HC-1(R) and [D]HC-1(R) reduce the number of viable cells in *S. aureus* biofilm, complete reduction is not observed. Interestingly, considerable activity against *E. coli* biofilm is observed only for [D]hBTD-1, an analog of HBD-1 containing D-amino acids. The analog [D]hBTD-1 shows comparable activity against *E. coli* and *S. aureus* biofilms with a MBEC of 200 μ M. Clearly, not all analogs eradicate biofilms to the same extent. Linear fatty acylated analogs, disulfide constrained analogs and chimeric analogs show comparable potencies against planktonic forms of bacteria. It appears that conformational constraints by a disulfide bond appears to play a critical role in endowing antibiofilm activity to defensin analogs. Interestingly, amino acid sequences of chimeric defensins hBTD-1 and [D]hBTD-1 differ from HC-1(R) and [D]HC-1(R) only by the C-terminal segment. This

segment of chimeric defensin analogs "KAKRIGRRIC" has more cationic residues than the corresponding segment "RAKCR" of HC-1(R). Further, it also contains hydrophobic residues. Presumably, the combination of hydrophobicity and cationicity in the "KAKRIGRRIC" sequence facilitates stronger penetration of peptides into biofilm matrix and improves the killing efficacy. Unlike the activity against, *S. aureus* biofilms, peptide backbone chirality appears to play critical role in the activity against *E. coli* biofilm. The inability of [D]HC-1(R), a peptide with several D-amino acids, to eradicate *E. coli* biofilm suggests that mere proteolytic stability cannot account for the efficacy of [D]hBTD-1. Our study provides significant insights into the structural requirements for disrupting pre-formed biofilms in human defensin analogs. Further, D-enantiomers of defensins could become potential candidates for treating biofilm-associated infections.

Variations in the Interaction of Human defensins with *Escherichia coli*: Possible Implications in Bacterial Killing

Human α and β -defensins are cationic antimicrobial peptides characterized by three disulfide bonds with a triple stranded β -sheet motif. Despite having similar structures, mammalian α - and β -defensins show considerable variations in their antibacterial potencies and spectrum of activity. The interactions by α - and β -defensins with model membranes are highly variable and their relevance to bacterial killing is not yet established unequivocally. It is presumed that interaction with the bacterial cell surface and membrane permeabilization by defensins is an important step in the killing process. The activities of α - and β -defensins on model membranes or membranes of bacteria have not been compared in the same series of experiments. This would help direct comparison of their activities and also get better insights into the differences in their interaction with membranes. We have compared membrane activities of four human α -defensins and four β -defensins against bacterial and model membranes. We have also included the inactive α -defensin HD6 in the study. Among the α -defensins, HNP4, HD5 and HD6 were more effective in increasing the zeta potential as compared to HNP3.

Among the β -defensins, HBD1 was the least effective in increasing the zeta potential. The zeta potential modulation data indicate variations in the surface charge neutralizing ability of α - and β -defensins. Comparison of *E. coli* inner membrane and model membrane permeabilizing abilities indicated that HD5, HD6 and HBD1 do not permeabilize membranes. Although HBD4 does not permeabilize model membranes, considerable damage to the inner membrane of *E. coli* is observed. Thus, there are considerable variations in their ability to interact with the *E. coli* cell surface and model membranes, suggesting differences in the mechanisms by which human defensins exert their antibacterial activity. Although our arguments are based on the

observations on *E. coli*, similar variations in the mechanism of bacterial killing can be anticipated for other gram-negative species as well. Defensins show differences in membrane destabilization which could result in variations in bacterial killing mechanisms despite having very similar three dimensional structures. It is evident that the topography of positively selected amino acids during the evolution play a critical role in rendering highly heterogeneous mechanisms of bacterial killing without affecting their overall three dimensional fold. In conclusion, our study indicates that mammalian defensins do not kill *E. coli* by a simple mechanism involving membrane permeabilization though their antibacterial potencies are very similar.

Palani Murugan Rangasamy

The Regulation of Polyamine Homeostasis and their relevance in Health and Diseases of Eukaryotes



From left to right : Archana Gopalakrishna, Babu R, Ashis Kumar Pradhan, Palani Murugan Rangasamy, Upasana Chatterjee, Shravani Kandikanti, Alavelu

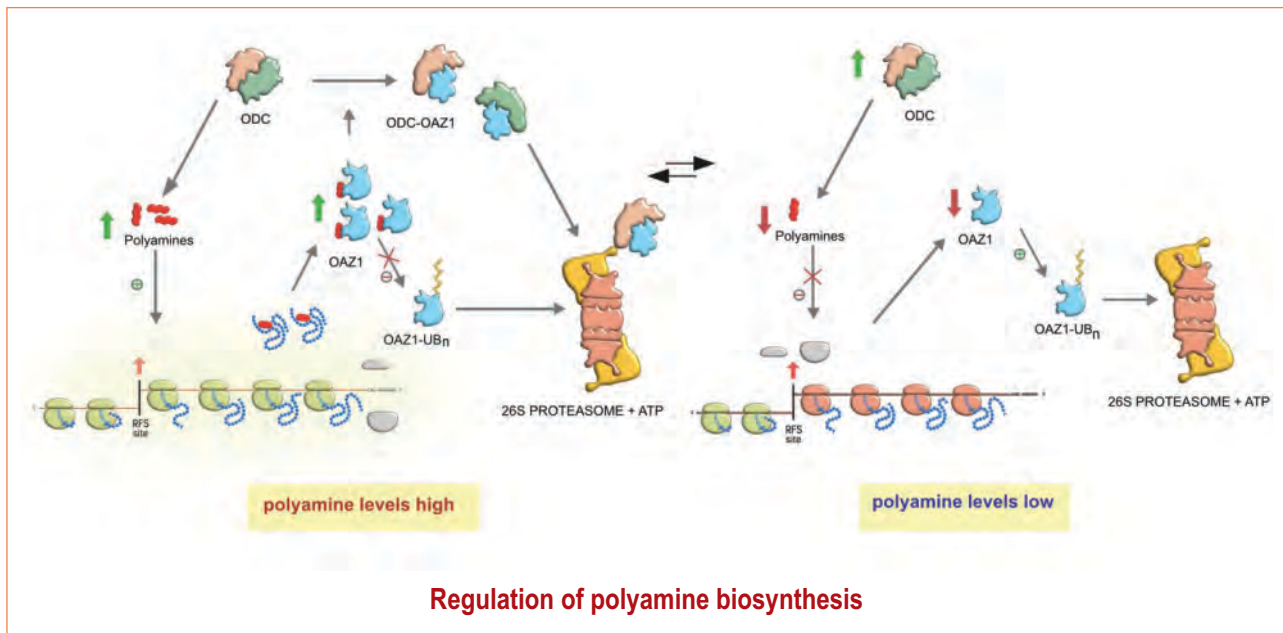
RESEARCH INTERESTS:

- "The research group is interested in understanding the regulation of polyamine homeostasis and their relevance in health and diseases of eukaryotes"

Our research team is understanding the basic biology of polyamine homeostasis and their relevance in human health and disease.

Selected recent publications

1. Beenukumar R, Gödderz D, Palanimurugan R, Dohmen RJ (2015) Polyamines directly promote antizyme-mediated degradation of ornithine decarboxylase by the proteasome. *Microbial Cell* 2(6): 197-205.
2. Kurian L, Palanimurugan R, Gödderz D, Dohmen RJ(2011). Polyamine sensing by nascent ornithine decarboxylase antizyme stimulates decoding of its mRNA. *Nature* 477(7365): 490-94.
3. Gödderz D, Schäfer E, Palanimurugan R, Dohmen RJ (2011) The N-terminal unstructured domain of yeast ODC functions as a transplantable and replaceable ubiquitin-independent degran. *J Mol Biol.* 407(3):354-67.
4. Palanimurugan R, Scheel H, Hofmann K, Dohmen RJ(2004). Polyamines regulate their synthesis by inducing expression and blocking degradation of ODC antizyme. *EMBO J.* 23(24): 4857-67. *Highlighted in:Complex regulation by polyamines of their own synthesis. Nature view. 2004 Nov 18.*
5. Marques AJ, Palanimurugan R, Matias AC, Ramos PC, Dohmen RJ.(2009) Catalytic mechanism and assembly of the proteasome. *Chem Rev.* 109(4): 1509-36.



Polyamines are aliphatic, multivalent polycations having various cellular functions. Although many derivatives are present putrescine, spermidine and spermine are namely the most important polyamines. Because of their diversity of functions, they are essential for life and found in all life forms. Polyamines are indispensable for normal cellular growth, they prolong lifespan, act as odour, and donate modifying group for hypusination. Due to their property in stimulating growth, higher levels of polyamines are shown to cause cancer and lower levels of polyamines induce apoptosis or cell death. Further, imbalances in polyamine levels are also associated with aging and neuro-degeneration. Regulating polyamine homeostasis therefore is critical for normal

cell and organismal physiology. In an evolutionarily conserved mechanism from yeast to humans, polyamine biosynthesis is regulated by a feedback mechanism that controls the rate-limiting enzyme Ornithine Decarboxylase (ODC) by ODC antizyme (OAZ or AZ). Using unicellular as well as multi cellular model systems our research work is aimed at understanding this important process.

Research questions

1. How are polyamine levels sensed in the cell?
2. What are the molecular mechanisms of polyamine homeostasis?
3. What is the effect of deregulated polyamine homeostasis in human health and disease?

Ch Mohan Rao

Molecular Chaperones in Health and Diseases, Molecular Diagnostics, Therapeutics and Drug Delivery



From left to right (top): Ch. Mohan Rao, (Bottom) Kranti Kiran Akula, Prashanth Budnar, Kamakshi Dandu, Gopi Suresh Oggu

RESEARCH INTERESTS:

- We combine molecular and cell biological approaches as well as nanobiology and microfluidics to address problems of biomedical importance. Our main objectives include understanding the structural and functional aspects of heat shock proteins in health and disease and the development of affordable molecular diagnostics and point-of-care devices.
- Molecular chaperones and heat shock proteins
- Molecular diagnostics and point-of-care devices
- Nanobiology and microfluidics
- Stem cells and angiogenesis

Our laboratory has been investigating, on one hand, molecular chaperones and small heat shock proteins in the context of health and disease, and on the other hand developing therapeutics for AMD (Age-related Macular Degeneration), diabetic retinopathy, retinoblastoma, smart nano drug delivery systems.

Selected recent publications

1. Ahsan SM, and Rao CM (2017) Condition responsive nanoparticles for managing infection and inflammation in keratitis. *Nanoscale*, 9 (28); 9946-9959(Covered in "Chemistry World" a monthly chemistry news magazine published by the Royal Society of Chemistry, UK).
2. Ahsan SM and Rao CM (2017) Role of surface charge in the desolvation process of gelatin: implications in nanoparticle synthesis and modulation of drug release *Int. J. Nanomedicine*, 12; 795–808.
3. Ahsan SM and Rao CM (2017) Structural studies on aqueous gelatin solutions: implications for designing a thermo-responsive nanoparticulate formulation *Int. J. Biological Macromolecules*, 95; 1126-1134.
4. Suvarsha Rao M, Ramakrishna T and Rao CM (2017) Clusterin: full-length protein and one of its chains show opposing effects on cellular lipid accumulation *Scientific Reports*, 7: 41235.
5. Kiran Kumar B, Gopi Suresh O, SasiKumar S, Nirosha R, Rao CM, Kranthi Kiran R(2017): Gene delivery approaches for mesenchymal stem cell therapy: Strategies to increase efficiency and specificity. *Stem Cell Reviews and Reports*: doi 10.1007/s12015-017-9760-2.

Heat Shock Proteins and Molecular Chaperones in Health and Disease

Heat shock proteins (Hsps) and molecular chaperones are important in several cellular processes. We demonstrated that the eye lens proteins, α A- (HspB4) and α B-crystallin (HspB5) and their hetero-oligomeric form function as molecular chaperones, prevent aggregation of non-native proteins by providing appropriately placed hydrophobic surfaces. Our study also showed that a temperature-induced structural transition enhances the chaperone activity. This finding opened up the possibility of mitigating complications arising out of protein misfolding and aggregation by enhancing chaperone-like activity. We engineered chimeric proteins with several fold higher activity with potential therapeutic applications. We are presently investigating the effect of α B-crystallin and its phosphorylation on angiogenesis, amyloidogenesis and on stem cell behavior. Our studies also include other sHsps such as Hsp27 (HspB1), HspB2, HspB3, HspB8 and extracellular chaperones such as clusterin and haptoglobin. We have demonstrated that α A-crystallin, α B-crystallin and other sHsps such as Hsp27, HspB2 and HspB3 bind to copper and confer cytoprotection.

Role of α B-crystallin(HspB5) and its phospho-mimicking mutants in angiogenesis

(Kiran Kumar Bokara, T. Ramakrishna, Ch.Mohan Rao)

HspB5 is shown to promote angiogenesis by interacting with VEGF. HspB5 is phosphorylated at three serine residues, Ser-19, Ser-45, and Ser-45

under different conditions. In order to investigate the role of phosphorylation in angiogenesis, we have generated single-, double- and triple-phosphorylation-mimicking mutants. The expressed proteins were characterized using circular dichroism, intrinsic fluorescence, bis-ANS binding, dynamic light scattering, and analytical ultracentrifugation. Phospho-mimicking mutant proteins were used to evaluate vascular development using chick chorioallantoic membrane (CAM) assay and Tube formation assay using human retinal microvascular endothelial cells (HRMVECs). Our results showed the internalization of exogenously added FITC-labeled HspB5, and its phospho-mimics in the HRMVECs (Fig. I). HspB5 as well as the phospho-mimicking mutants induced morphological changes such as the formation of filopodia or lamellipodia (Fig. I). Moreover, the CAM assay results showed that treatment with HspB5 and its phospho-mimics significantly induced new blood vessel formation as monitored by the increase in the length, size and branching of blood vessels compared to the PBS-treated controls (Fig. II). In conformity with the results of the CAM assay, similar treatment of HRMVECs grown on Matrigel™ resulted in enhancement of tube formation and branching (Fig. III), suggesting a role for HspB5 and its phospho-mimics in angiogenesis. Phosphorylation-mimicking mutants differed in their capacity to induce different angiogenic parameters. These observations should be of use in developing alternative therapeutic strategies, such as inhibiting HspB5 and/or disrupting the interaction between VEGF and HspB5, to manage neovascularization-related eye diseases.

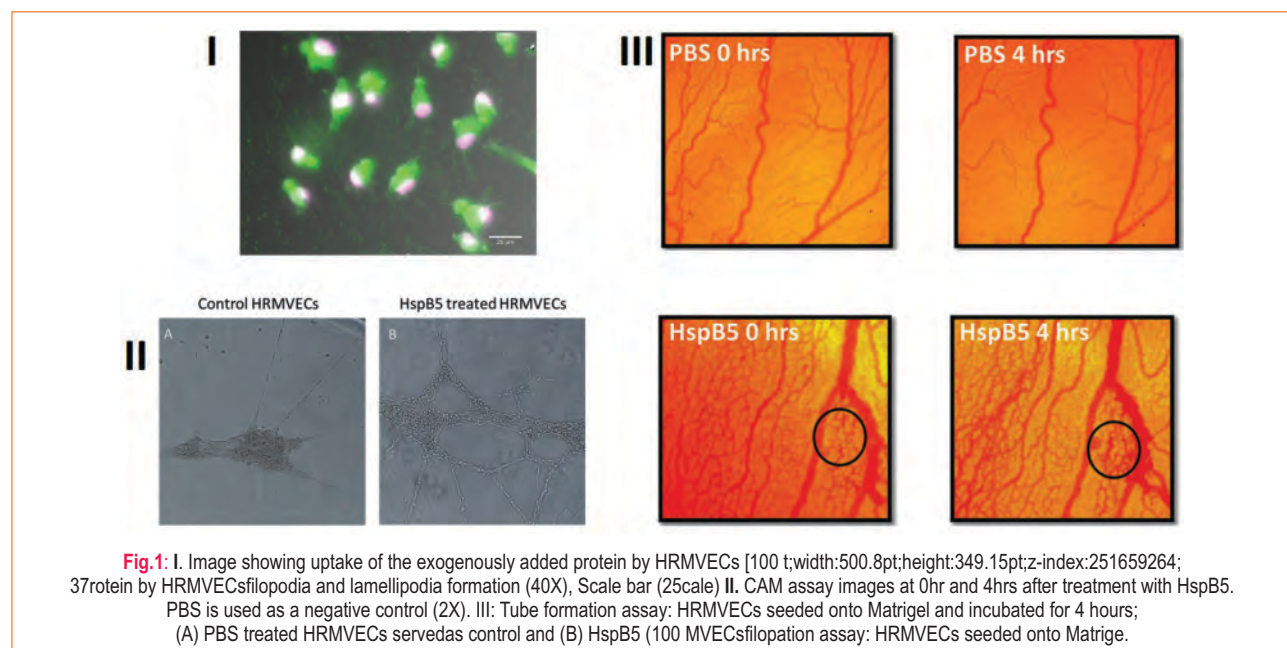
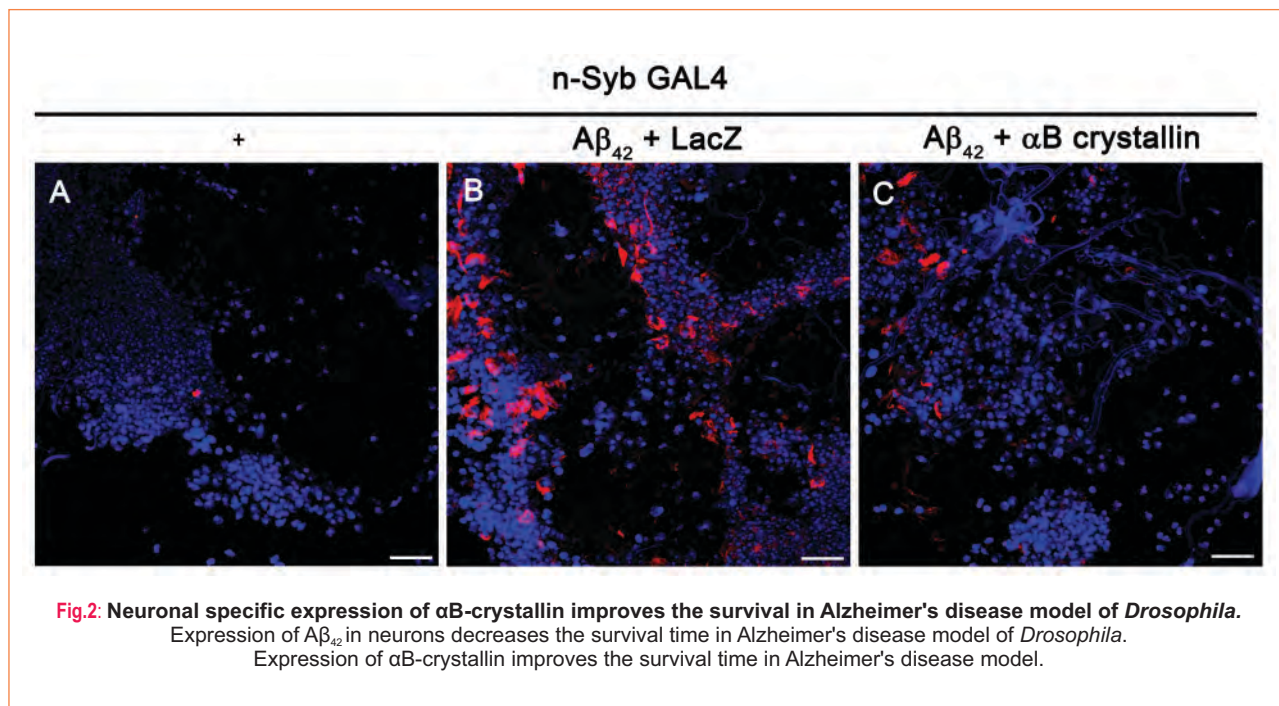


Fig. 1: I. Image showing uptake of the exogenously added protein by HRMVECs [100 t; width:500.8pt; height:349.15pt; z-index:251659264; 37protein by HRMVECs filopodia and lamellipodia formation (40X), Scale bar (25scale) II. CAM assay images at 0hr and 4hrs after treatment with HspB5. PBS is used as a negative control (2X). III: Tube formation assay: HRMVECs seeded onto Matrigel and incubated for 4 hours; (A) PBS treated HRMVECs served as control and (B) HspB5 (100 MVECs) filopodia assay: HRMVECs seeded onto Matrige.



Alpha B Crystallin (HspB5) in neurodegenerative diseases:

(Rakesh Mishra and Ch. Mohan Rao)

Studies from several laboratories including ours have shown that αA - and αB -crystallin would be useful in protecting protein aggregation in diseases such as Alzheimer's, Parkinson's and Huntington's disease. In collaboration with Dr. Rakesh Mishra's group, we are using *Drosophila* as an *in vivo* model to test the role of α -crystallins in neurodegenerative diseases/protein aggregation diseases. Previously, we showed that αB -crystallin expression reduces the rough eye phenotype in Alzheimer's disease model of *Drosophila*. During this year, we have found that

αB -crystallin expression in neurons of Alzheimer's disease model increases the survival time. The mean life span of flies expressing $A\beta_{42}$ is found to be 25.8 days and that of flies expressing $A\beta_{42}$ along with HspB5 increased to 33.4 days (Fig 1).

In addition, the quality of life also improved significantly. HspB5 expression rescues locomotory defects caused by $A\beta_{42}$ aggregation (Fig 3). The observed increase in lifespan and improved locomotor function upon HspB5 expression appears to be due to its ability to reduce aggregation of $A\beta_{42}$ (Fig 2).

Mutant human αA -crystallin and congenital cataract:

(T. Ramakrishna and Ch. Mohan Rao)

αA -crystallin plays an important role in eye lens development. It has three distinct domains viz. the N-terminal, α -crystallin domain and the C-terminal extension. Certain arginine residues in the N-terminal domain of αA -crystallin appear to be conserved across the sHsp family. Most of the cataract-causing mutations in αA -crystallin have been reported to be in these conserved arginine residues, highlighting their role in the functionality of αA -crystallin. During the year, we have attempted to understand the molecular basis of cataract resulting from the mutation of arginine-54 residue to cysteine in αA -crystallin. The mutation led to minimal changes in the secondary and tertiary structure of αA -crystallin with no observable changes in the chaperone-like activity. However,

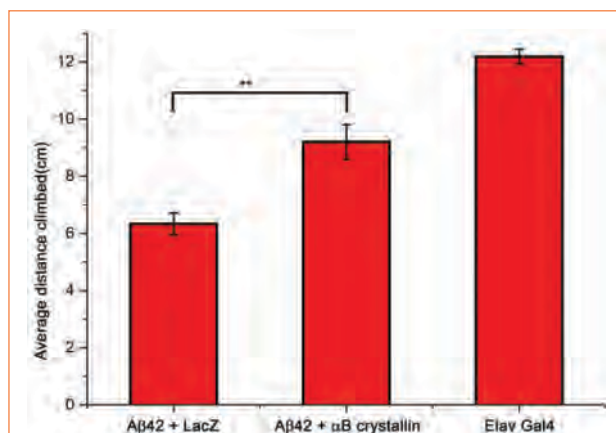


Fig.3: Expression of αB -crystallin in neurons decreases the $A\beta_{42}$ aggregates in Alzheimer's disease model of *Drosophila*. (A) GAL4 driver control; (B) Immunohistochemistry with $A\beta_{42}$ specific antibody shows $A\beta_{42}$ aggregates (in red) in 15-day old flies which coincides with behavioral defects; (C) αB -crystallin expression decreases the $A\beta_{42}$ aggregates. Scale bar is 25 μ m

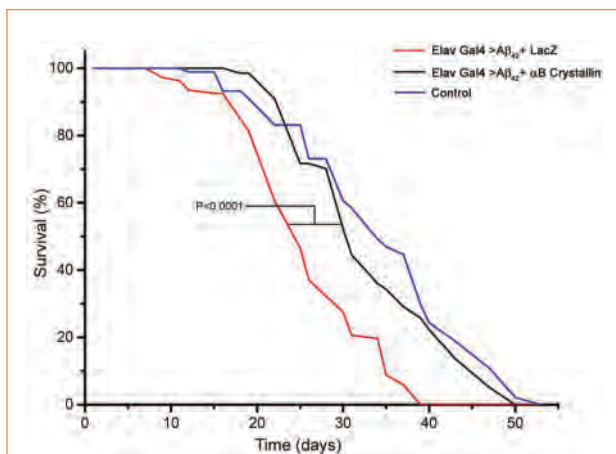


Fig.4: Expression of α B-crystallin in neurons decreases the $A\beta_{42}$ aggregates in Alzheimer's disease model of *Drosophila*. (A) GAL4 driver control; (B) Immunohistochemistry with $A\beta_{42}$ specific antibody shows $A\beta_{42}$ aggregates (in red) in 15-day old flies which coincides with behavioral defects; (C) α B-crystallin expression decreases the $A\beta_{42}$ aggregates. Scale bar is 25 μ m

when expressed in lens epithelial cells, R54C- α A-crystallin aggregated and translocated to the nuclei of the cells, causing a stress-like response, and translocation of α B-crystallin to the nucleus. The mutation also led to a decrease in its interaction with HSPB5 and HSP27 and triggered cell death through apoptosis, as evident by the activation of Caspase 3. Our results shed light on the molecular basis of cataract caused by the R54C mutation in α A-crystallin and highlight the important role of altered inter-protein interactions in the pathogenesis.

Retinoblastoma:

(Suman Thakur and Ch. Mohan Rao)

Retinoblastoma, ocular cancer, is mostly associated with early childhood. Many cases need enucleation of the eye. If not treated in time, it is fatal due to intracranial extension and metastasis. We observed that a small molecule disrupts clusters of retinoblastoma cell line Y79. These cells grow in clusters and disruption of clusters leads to cell death. By performing proteomics, we identified that a large number of cell adhesive molecules are involved in the process. Interestingly, we also observed that oncogene products are down-regulated and several anti-proliferative factors are up-regulated upon treatment with this small molecule. The molecule was codenamed E4 for its potential patentability.

Cell culture studies showed that E4 is effective in killing Y79 cells. We extended our studies to *in vivo* models. Xenografted nude mice of Y79 cells developed tumors. Treatment of these tumors with E4 resulted in dramatic regression of the tumors. Histopathological evaluation showed that

treatment with E4 did not affect liver, spleen, lung, kidney and heart.

Encouraged by the results of our xenograft nude experiments, we planned to test the molecule in a retinoblastoma genetic knockout mouse model (Chx10-Cre; RbLox^{-/-}; p53Lox^{-/-}; p107^{-/-}). Due to the absence of Rb, p53 along with p107, these knockout mice develop aggressive retinoblastoma. During the year, we have obtained knockout animals and are breeding them at our animal facility. The increase in the colony size is very slow due to the high mortality rate of the animals. We have initiated studies with intra-tumoral injections. We believe that this non-toxic molecule, with its low molecular weight and good water solubility would be a potential therapeutic for retinoblastoma.

Paper-based microfluidic devices:

Early diagnosis significantly reduces the health care burden and also improves the treatment outcome. Our country needs affordable, scalable diagnostics that can be used by unskilled persons in semi-urban and rural settings; preferably not requiring the cold chain. Paper microfluidics-based, point-of-care systems are eminently suitable for our needs. We have built the capacity to design and develop such paper microfluidic systems. We are now developing paper-based microfluidic devices for blood typing, determination of Erythrocyte Sedimentation Rate, pregnancy detection kit for livestock etc. We are also working towards developing DNA-based diagnostics for human diseases on a paper-based system.

Paper-based microfluidic viscometer:

(Amit Asthana and Ch. Mohan Rao)

During our experiments with a paper-based ESR device, we noticed that capillary mobility of liquids in paper-based device clearly depended on their viscosity. Based on this observation, we set out to develop a paper-based viscometer. We successfully designed a device with required stability, measured viscosities of a few test fluids and obtained an excellent correlation coefficient of 0.9997 with the results obtained using a conventional Ostwald's viscometer. A paper-based viscometer offers advantages such as time efficiency, cost-effectiveness, and low sample volume requirement over the conventional viscometer. These paper-based devices can also be used to estimate molecular weights of polymers with reasonable accuracy.

Surface Enhanced Raman Spectroscopy (SERS) for enhancing the sensitivity of biomarker detection.

(Amit Asthana and Ch. Mohan Rao)

Raman spectroscopy, despite the use of LASERS, is not very sensitive to detect very low concentrations of biomarkers. SERS is known to provide a significant enhancement in the signal. Hence, either a roughened surface or substances with small radii of curvature are used. We are investigating several different methods to deposit metals onto the paper device to use them as SERS substrates. During the year we successfully tested our substrates with lysozyme and Rhodamine 6G.

Development of paper-based pregnancy detection kit for cattle:

(Amit Asthana, G. Umapathy and Ch. Mohan Rao)

Early detection of pregnancy would help shorten the calving interval and plan for re-breeding. This leads to

maximizing the milk production, thereby enhancing the farmer's income (The value of the dairy industry is Rs. 5 lakh crores in 2015 and reported to be growing). Dr. Umapathy's group identified a metabolite that correlates with the pregnancy status in wild animals. They observed that the metabolite could also be used for determining the pregnancy status in cattle. Based on these observations we set out to develop a paper-based device for an early pregnancy test in cattle. During the year, we have troubleshooted several issues with antibody, stability, different methods of detection etc. and made a few prototypes with encouraging results. Presently, we are also developing another antibody and different detection strategies to improve stability and sensitivity. The device would be usable by untrained persons with the naked eye and would not require a cold chain.

Swasti Raychaudhuri

Proteotoxicity in Age-related Diseases



From left to right : Swathi, Shivali, Anusha, Swasti, Bodhisattwa, Debodyuti, Shemin

RESEARCH INTERESTS:

- How do misfolded proteins promote proteotoxicity?
- Why are protein-misfolding diseases prevalent during aging?

- Generated multiple cell culture based protein aggregation models - mild to aggressive
- Developed multiple quantitative mass spectrometry based assays to investigate protein destabilization events in mass-scale
- Shown proteasome dysfunction directly impedes mitochondrial respiration by perturbing stability and assembly of Respiratory Complex subunits, and provide an explanation for the association between these two hallmarks of age-related degenerative diseases.

Selected recent publications

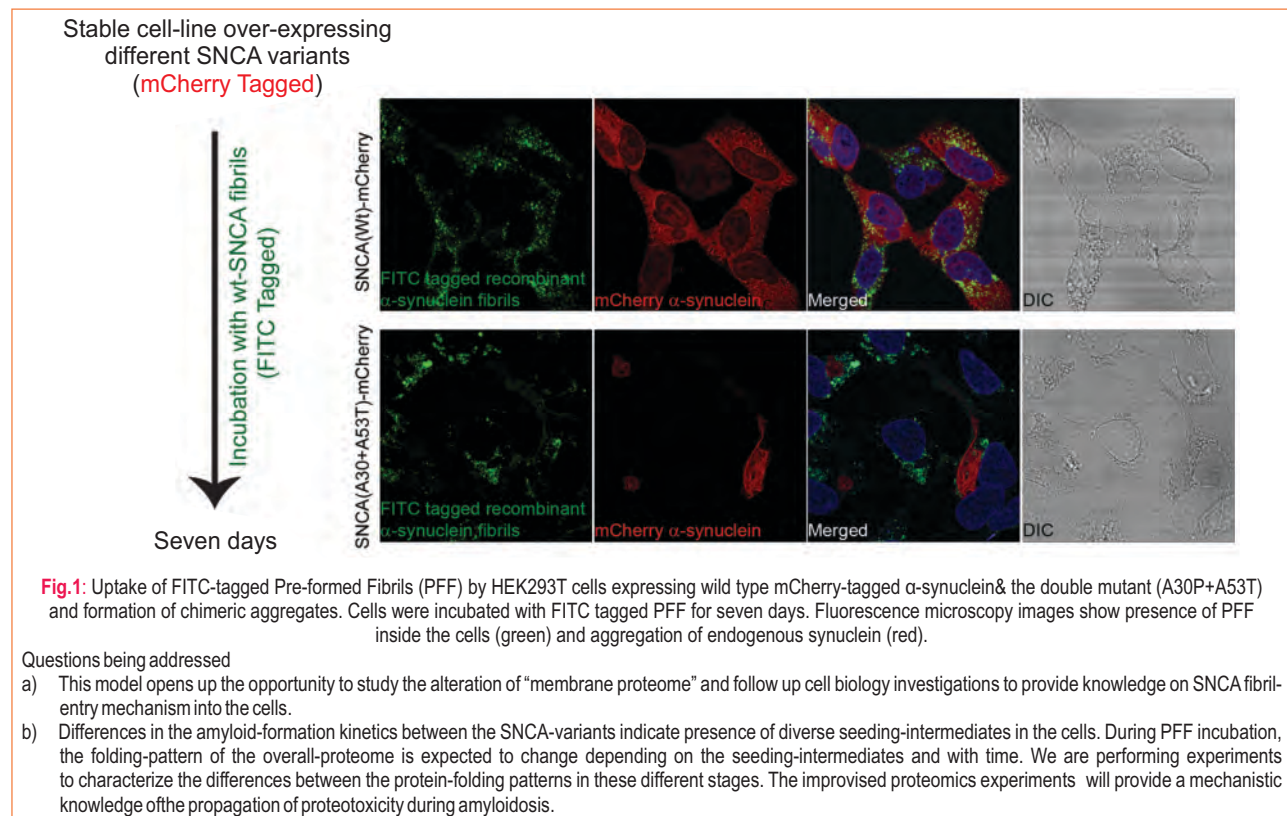
1. Raychaudhuri S, Banerjee R, Mukhopadhyay S, Bhattacharyya NP (2014) Conserved C-terminal nascent peptide binding domain of HYPK facilitates its chaperone like activity. *J. Biosci* 39:1-14.
2. Raychaudhuri S, Loew C, Koerner R, Pinkert S, Theis M, Hayer-Hartl M, Buchholz F, Hartl FU (2014) Interplay of Acetyltransferase EP300 and the Proteasome System in Regulating Heat Shock Transcription Factor 1. *Cell* 156(5):975-85.
3. 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. *Nat Methods* 8(10):879-84.

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

Cancer, diabetes, cardio-vascular diseases and several neurodegenerative disorders represent the majority of the protein-misfolding related health-setbacks in the aging population. For example, in each of the neurodegenerative disorders known, a "symptomatic-protein" misfolds and damages the protein homeostasis in the nerve cells in various ways. Formation of toxic aggregates of the islet amyloid polypeptide (IAPP) contributes to β -cell dysfunction and diabetes. Soluble pre-amyloid oligomers are present in the cardiomyocytes of many human heart failure samples. Chronic, uncontrolled protein misfolding and resultant abnormal heat shock protein load is known to foster tumor development. Importantly, loss-of-function of the misfolded proteins is not likely to be the major reason behind proteotoxicity. Rather, several common protein-metabolic pathways (starting from protein synthesis,

folding, transport to degradation) are perturbed in all these diseases. This common pathogenic modality offers unique opportunity to discover novel drug-targets that could be useful in ameliorating multiple proteotoxic conditions; however, systemic investigations are emergent.

In our lab, we are interested in studying the impact of symptomatic misfolding-prone protein on the cellular proteome using different protein-misfolding disease models. The novelty of our approach relies on the identification of exclusive molecular players which could be targeted in multiple diseases. Currently, we are using mutant versions of α -synuclein (responsible for familial Parkinson's disease), Ubiquitin-2 (Amyotrophic Lateral Sclerosis), Huntingtin (Huntington's disease) and FlucDM-EGFP [1] as model misfolding-prone proteins. Several cell-lines conditionally expressing these misfolding-prone proteins have been generated. Multiple chemical biology tools and quantitative proteomics protocols to study the proteostasis changes in these cell-lines have been successfully standardized. Pilot experiments to study the impact of protein-misfolding on the soluble and functional proteome, activation of stress response[2] and protein degradation etc. have been initiated. These investigations will lead to: a) Understanding the mechanism of proteotoxicity in protein-misfolding diseases; b) The identification of novel drug targets; c) The development of multi-dimensional, high-throughput small-molecule screening platforms for protein-misfolding diseases.



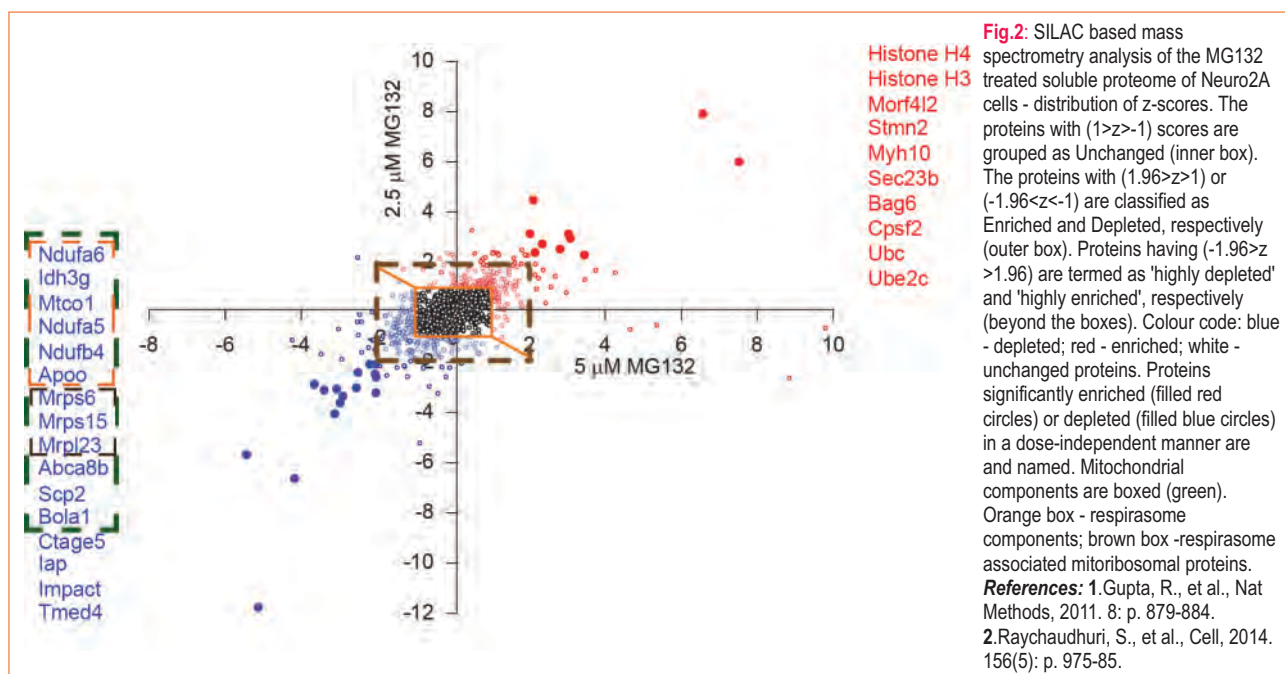
Proteotoxicity by α -Synuclein

To understand the mechanism of proteotoxicity caused by α -Synuclein, we are using α -Synuclein (SNCA) expressing cell-culture models. Duplications and triplications of this gene as well as mutations in the N-terminal region (A53T, A30P, E46K etc.) cause aggregation of this protein resulting in a range of synucleinopathies. 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 probably 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.

Re-adjustment of the functional proteome in response to proteasome-block

Proper folding and solubility are two major determinants of protein functions. A large number of proteins deviate from their native functions due to misfolding and insolubility in age-related neurodegenerative diseases. Simultaneous proteasome-dysfunction often increases the load of

non-functional ubiquitinated proteins resulting in an accumulative proteotoxicity. We blocked protein-degradation in Neuro2A cells with MG132; a specific cell-permeable proteasome inhibitor. Using a low dose and early incubation time, we studied the primary-reorganization events in the soluble proteome. Proteasome inactivation resulted in loss-of-solubility of several mitochondrial respirasome components as measured by SILAC-based quantitative mass spectrometry. These depleted proteins were mostly nuclear encoded and rely on chaperones to safeguard their loosely-folded conformation during translocation. Functional impairment of these proteins marks the onset of mitochondrial dysfunction during proteasome-inhibition. High chaperone-demand by these proteins may be responsible for titrating out heat shock proteins from the dormant chaperone-Hsf1 complex to trans-activate heat shock response as apparent by the elevated mRNA levels of hsp70 (hspa1b) and ubiquitin (ubc). Moreover, we observed increased level of histone H3 and H4 proteins and their modifiers (Morf4I2) in the soluble proteome indicating fine-tuning of chromatin-assembly. Histone-H3 trimethylation (H3K4me3) level was also increased which is a known prerequisite for successful Hsf1-mediated transcription. Thus, we report the reorganization events in the soluble proteome of Neuro2A cells at an early juncture that allows predicting the long term toxic-consequences of proteasome-inhibition.



Manjula Reddy

Bacterial Cell Wall Synthesis and its Regulation



From left to right (Front row): Aisha Hamid*, Shambhavi Garde, Sadiya Parveen, Anju Bala, Moneca Kaul, L Sai Sree
(Back row) Raj Bahadur, Manjula Reddy, Pavan Kumar, AnubhavBharadwaj, V Balaji, NilanjanSom, Pratap Acharya* (Dissertation students*)

RESEARCH INTERESTS:

- Understanding cell wall biogenesis and its regulation in bacteria

Selected recent publications

1. Parveen S and Reddy M (2017) Identification of YfiH (PgeF) as a factor contributing to the maintenance of bacterial peptidoglycan composition. *Molecular Microbiology* doi: 10.1111/mmi.13730.
2. Singh SK, Parveen S, SaiSree L and Reddy M (2015) Regulated proteolysis of a cross-linkspecific peptidoglycan hydrolase contributes to bacterial morphogenesis. *Proc. Natl. Acad. Sci., USA* 112: 10956-10961.
3. Mahalakshmi S, Sunayana MR, SaiSree L and Reddy M (2014) *yciM* is an essential gene required for regulation of lipopolysaccharide synthesis in *Escherichia coli*. *Molecular Microbiology* 91: 145-157.
4. Singh SK, SaiSree L, Amrutha RN and Reddy M. (2012) Three redundant murein endopeptidases catalyze an essential cleavage step in peptidoglycan synthesis of *Escherichia coli* K12. *Molecular Microbiology* 86: 1036-1051

Our recent results show that editing or proofreading pathways exist in bacteria to maintain the composition and integrity of bacterial peptidoglycan

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 the Gram-negative rod-shaped bacterium *Escherichia coli* as a primary model system. We expect that our work facilitates a better understanding of fundamental aspects of bacterial cell biology and will also provide novel strategies for development of antimicrobial therapeutics.

Most bacteria are surrounded by a protective mesh-like exoskeleton called peptidoglycan (PG or murein) sacculus to protect them-selves from internal turgor pressure. PG is a single, large, covalently cross-linked macromolecule that forms a net-like structure completely encasing the bacterial cytoplasmic membrane. Hence, growth of a bacterial cell is intimately coupled to expansion of murein sacculus and requires concerted activity of hydrolases that cleave the cross-links for insertion of new material and synthases that catalyze the cross-link formation. Although, conceptualized nearly five decades ago, the mechanism of such essential murein cleavage activity was not known earlier. To examine the significance of cross-link cleavage in PG growth, we identified three previously unknown murein hydrolytic enzymes, two (Spr and YdhO) belonging to the NlpC/P60 peptidase superfamily and the third (YebA) 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. In summary, we demonstrated that the step of cross-link cleavage is essential for bacterial PG growth and identify these endopeptidases to be the long-postulated space-maker hydrolases required for enlargement of the murein sacculus (Singh, Sai Sree, Amrutha and Reddy, *Molecular Microbiology*, 2012). Accordingly, these endopeptidases were renamed MepS (Spr), MepM (YebA) and MepH (YdhO), respectively, in which *mep* stands for *murein endopeptidase*.

Although, the cross-link cleavage is fundamental for PG growth, such cleavage needs to be stringently regulated at the spatiotemporal level to avoid lethal breakage and rupture of PG. To understand how this potential lethal hydrolytic activity is controlled in the cell, we examined the regulation of the D, D-endopeptidase, MepS. We find that expression of MepS is dependent on bacterial growth cycle; it is highly abundant in exponential phase of growth with the levels falling sharply at the onset of

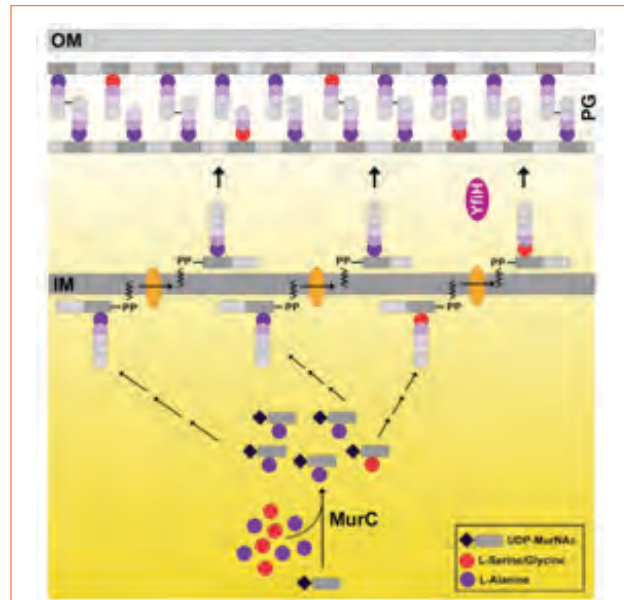


Fig.1: Schematic of peptidoglycan editing pathway by YfiH (PgeF). Peptidoglycan (PG) precursors are synthesized in the cytosol and are flipped across the inner membrane (IM) to be incorporated into the existing PG sacculus. YfiH edits the incorrect muropeptides in the periplasmic space (OM-Outer membrane).

stationary phase. Using combined genetic, molecular and biochemical approaches, we showed that MepS is rapidly degraded with a half-life of ~2 min by a novel proteolytic system comprising of an outer membrane (OM) lipoprotein of unknown function, Nlpl, and a periplasmic protease, Prc. In summary, we showed that Nlpl-Prc system modulates PG synthesis by regulating the levels of MepS, a hydrolase that breaks the cross-links for insertion of new murein material during growth of PG sacculus. MepS is the only enzyme of PG metabolism to be regulated during growth cycle signifying the importance of cross-link cleavage suggesting that the step of cross-link cleavage may indeed be the rate-limiting step of PG biosynthesis (Singh, Sadiya, SaiSree and Reddy, *Proc. Natl.Acad. Sci.* 2015).

Recently, we showed that a conserved open reading frame of unknown function, YfiH (renamed PgeF for Peptidoglycan editinG Factor) contributes to the maintenance of peptide composition in *E. coli*. Using genetic, biochemical and mass spectrometrical analyses, we demonstrated that absence of *yfiH* results in incorporation of non-canonical amino acids, L-serine or glycine in place of L-alanine in PG sacculi leading to β -lactam sensitivity, lethality in mutants defective in PG remodelling or recycling pathways, altered cell morphology and reduced PG synthesis (Fig. 1). *yfiH* orthologs from other Gram-positive genera were able to compensate the absence of *yfiH* in *E. coli* indicating a conserved pathway in bacterial kingdom. Overall, our results suggest editing/ quality control mechanisms exist to maintain composition and integrity of bacterial peptidoglycan (Parveen and Reddy, *Molecular Microbiology*, 2017).

Regalla Kumaraswamy

Non-coding RNAs in Patho-physiology of the Heart



From left to right: Abishek Bharadwaj, Manisha Sahu, Regalla Kumaraswamy

RESEARCH INTERESTS:

- Non-coding RNAs
- Cardiac hypertrophy and failure
- Cardiac regeneration

The main focus of our newly established lab is to understand the role of non-coding RNAs in patho-physiology of the heart

Selected recent publications

1. Viereck J, Kumaraswamy R..... and Thum T(2016) Long noncoding RNA Chast promotes cardiac remodeling. *Science Translational Medicine* 8(326):326ra22.
2. Kumaraswamy R, Volkmann I et al and Thum T (2014) Vascular importance of the miR-212/132 cluster. *European Heart Journal* 35(45):3224-31.
3. Kumaraswamy R, Bauters C et al and Thum T (2014) Circulating long non-coding RNA, LIPCAR, predicts survival in patients with heart failure. *Circulation Research* 114(10):1569-75.
4. Kumaraswamy R, Lyon AR et al and, Thum T (2012) SERCA2a gene therapy restores microRNA-1 expression in heart failure via an Akt/FoxO3A-dependent pathway. *European Heart Journal*. 33(9):1067-75.
5. Kumaraswamy R, Volkmann I, Jazbutyte V, Dangwal S, Park DH, Thum T (2012) Transforming growth factor- β -induced endothelial-to-mesenchymal transition is partly mediated by microRNA-21. *Arteriosclerosis Thrombosis and Vascular Biology* 32(2):361-9

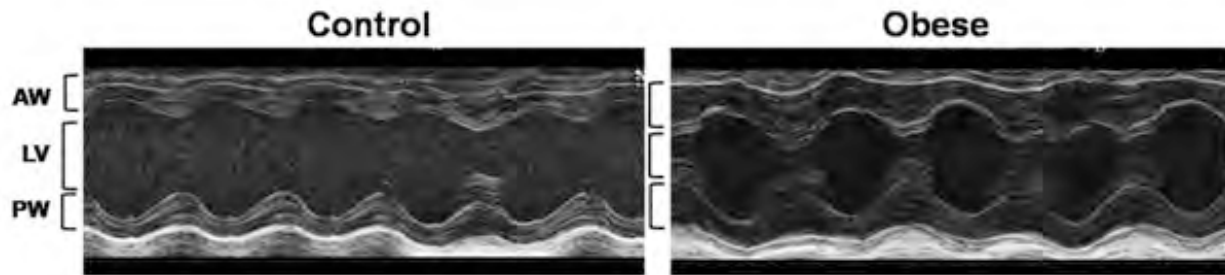


Fig.1: Left ventricular (LV) anterior (AW) and posterior (PW) wall thickness in control and obese mice were imaged by Vevo 2100 small animal ultrasound imaging system. Please note increased wall thickness in obese mice, which indicates left ventricular hypertrophy.

The human genome is tremendously larger than that of more simple organisms such as worms. However, genomes of humans and worms have a comparable number of protein coding genes. For instance, the genome of the 959cell bodied *C. elegans* and 100,000 billioncell bodied humans typically contain a similar number of protein coding genes and yet the human genome is 30 times bigger than the *C. elegans* genome (3000 MB vs 100MB). All protein coding genes in humans originate from about 2% of the genome and a majority of the remaining portion of the genome remains un-transcribed or transcribed as noncoding RNAs. All the available therapies and disease diagnostics today are based on this 2% of the coding genome. Until recently, the noncoding portion of the genome was believed to be 'junk' or dark matter of the genome and transcribed noncoding RNAs were described as 'Transcriptional noise'. However, recent studies have highlighted the importance of noncoding RNAs in various physiological and pathological settings and now they are referred as 'precise

regulators of gene expression'. MicroRNAs (~21 nt long) and long noncoding RNAs (≤ 200 nt long) are the two major classes of noncoding RNAs that regulate gene expression. Because of their gene regulatory function, noncoding RNAs have been proposed as attractive targets for development of novel therapeutic strategies in various disease conditions including heart failure.

Heart diseases are the leading cause of death worldwide. In India about 40% of all deaths in urban areas and 30% in rural areas are attributed to cardiovascular diseases. Incidence of heart diseases in India has steadily increased from about 2% (1960) to 10.5% (2000). Although available therapies improve symptoms, they are not able to reverse fibrosis or activate hibernating myocardium. The main research focus of our newly established lab in CCMB is to understand the role of non-coding RNAs in patho-physiology of the heart. Research in my lab is being funded by the Wellcome Trust/ DBT India Alliance.

Rajan Sankaranarayanan

Structural Biology



From left to right (1st row): Raghvendra, Aditi, Mazeed, Sankaranarayanan, Santosh, Murali, Rajkanwar, Ayshwarya, Shantiswaroop, Aditya, Jotin
(2nd row): Biswajit, Gajanan, Bhavita, Satya Brata, Priyadarshan, Priyanka, Rukmini, Lalitha, Sudipta, Mallesh, Aravind
(3rd row) : Subhash, Herojeet, Mantu Kumar, Suma, Sambavi, Shobha

RESEARCH INTERESTS:

- Proofreading during translation of the genetic code
- Mycobacterium tuberculosis complex lipid synthesis
- Secretome action of a rice pathogenic bacteria
- Calcium-binding proteins

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

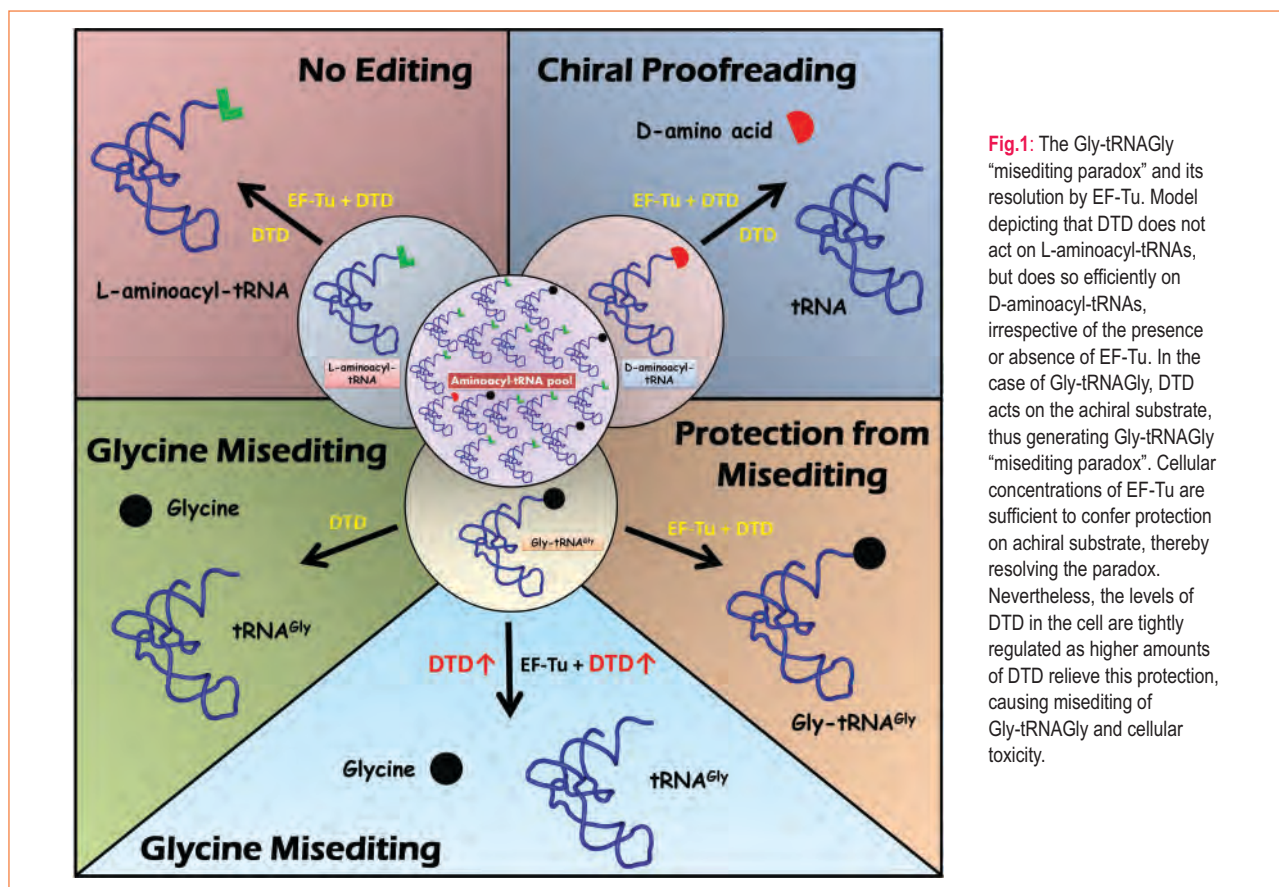
1. Srivastava SS, Jamkhindikar A A, Raman R, Jobby MK, Chadalawada S, Sankaranarayanan, R, Sharma Y(2017) A Transition Metal-Binding, Trimeric $\beta\gamma$ -Crystallin from Methane-Producing Thermophilic Archaea, *Methano saeta thermophila*. *Biochemistry* 59:1299-1310.
2. Routh, S B, Pawar K I, Ahmad S, Singh S, Suma K, Kumar M, Kuncha S K, Yadav K, Kruparani S P and Sankaranarayanan R (2016) Elongation factor Tu prevents misediting of Gly-tRNA (Gly) caused by the design behind the chiral proofreading site of D-aminoacyl-tRNAdeacylase. *PLoS Biol.* 14(5) e1002465, 1-22.
3. Kinatukara P, Patel KD, Haque AS, Singh R, Gokhale RS and Sankaranarayanan R (2016) Structural insights into the regulation of NADPH binding to reductase domains of nonribosomal peptide synthetases: A concerted loop movement model. *J Struct Biol.* 194:368-74.
4. Ahmad, S., Muthukumar, S., Kuncha, S.K., Routh, S.B., Yerabham, A.S., Hussain, T., Kamarthapu, V., Kruparani, S.P., Sankaranarayanan, R. (2015) Specificity and catalysis hardwired at the RNA–protein interface in a translational proofreading enzyme. *Nat Commun.* 6:7552.
5. Ahmad, S., Routh, S.B., Kamarthapu, V., Chalissery, J., Muthukumar, S., Hussain, T., Kruparani, S.P., Deshmukh, M.V., Sankaranarayanan, R. (2013) Mechanism of chiral proofreading during translation of the genetic code. *Elife* 2:e01519.

(highlighted by Science (343) Editor's Choice, "Chirality check", pp 119, 10th Jan, 2014).

We mainly use X-ray crystallography 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 employ various model organisms to establish the physiological relevance of some of these processes. A major focus of the lab is to study various factors involved in proofreading during translation of the genetic code. D-amino acids are occasionally attached to tRNAs by mistake. An enzyme named D-aminoacyl-tRNAdeacylase (DTD) hydrolyzes the ester bond between a D-amino acid and a tRNA, thereby helping to prevent infiltration of D-amino acids into the translational machinery. We have shown that DTD employs a Gly-*cis*Pro dipeptide motif in the active site to discriminate between L- and D-amino acids through strict L-chiral rejection rather than D-chiral selection. Consequently, DTD acts on both D-aminoacyl-tRNAs and the achiral substrate Gly-tRNA^{Gly}. This creates an unwanted Gly-tRNA^{Gly} “misediting paradox” which must be overcome because glycine is an integral component of proteins. The translational elongation factor thermo unstable (EF-Tu), which delivers aminoacyl-tRNAs to ribosome during protein synthesis, resolves the paradox by conferring protection on Gly-tRNA^{Gly}

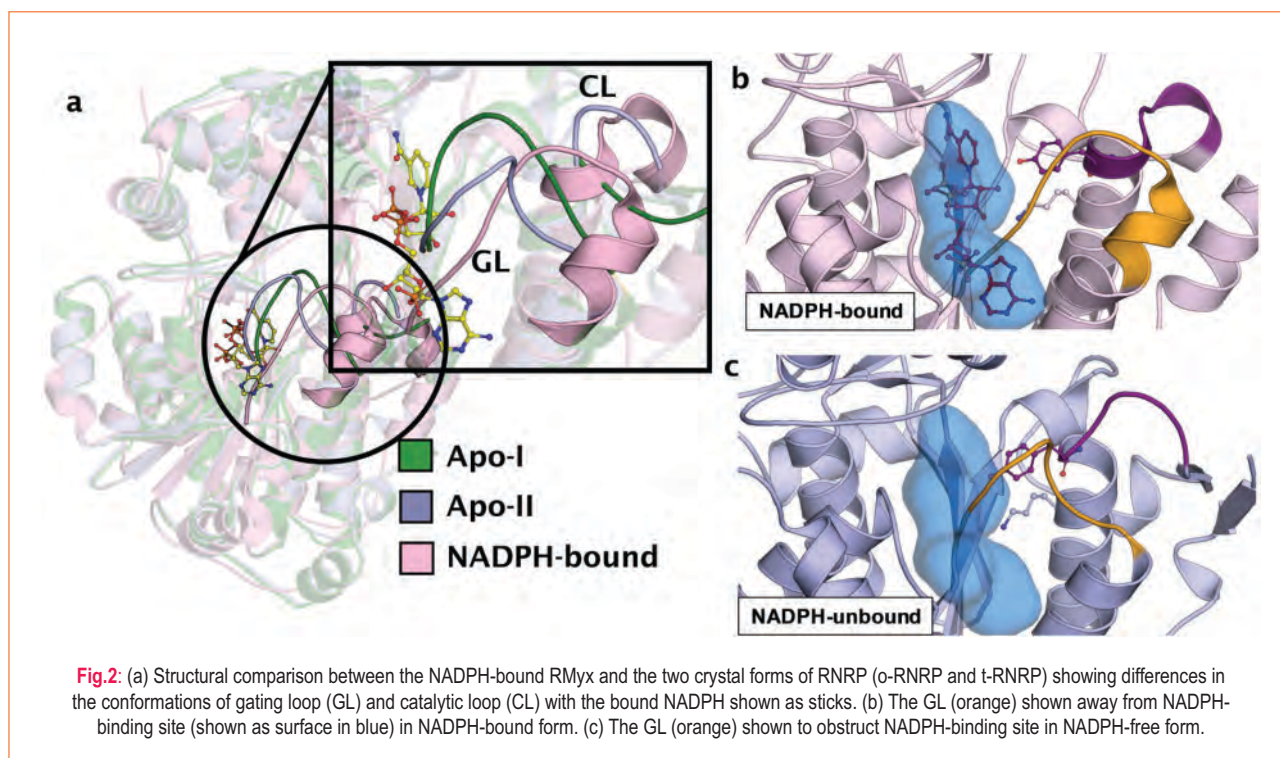
against DTD. However, DTD levels in cell must be kept low and tightly regulated because over expression of DTD causes cellular toxicity in *Escherichia coli* due to depletion of Gly-tRNA^{Gly}. We are currently probing DTD's role in eliminating the non-cognate (incorrect) Gly-tRNA^{Ala} species generated by alanyl-tRNA synthetase (AlaRS). We are also generating DTD knockout in the fruit fly (*Drosophila melanogaster*), zebrafish (*Danio rerio*) and mouse (*Mus musculus*) to decipher the physiological significance of DTD and its role in higher systems. We are also characterizing an Animalia-specific DTD homolog (ATD) and studying various aspects of other DTDs, namely DTD2 (present in archaea and plants) and DTD3 (found in cyanobacteria).

Another major project is on understanding the structure–function relationship of enzymes involved in the metabolism of complex lipids in *Mycobacterium tuberculosis* (Mtb) in collaboration with the group of Dr. Rajesh Gokhale, IGIB, New Delhi. Mtb lipids play a major role in its pathogenesis and are synthesized by polyketide synthases (PKS) and nonribosomal peptide synthetases (NRPS). Previously, our biochemical studies have revealed that Mtb uniquely employ reductases domains (R-domains) capable of



a reductive release of the biosynthetic products using the cofactor NADPH. The first crystal structure of R-domains from our laboratory revealed an additional C-terminal domain to the common Short-chain dehydrogenase/reductase fold, responsible for providing a hydrophobic platform for the final lipid product in Mtb. Our earlier Small-angle X-ray scattering (SAXS) studies revealed the process of binding of NADPH reorientation at the unique C-terminal domain. Structural comparison of two different crystal forms of apo R-domains showed significant differences in the C-terminal domain orientation and two important loops of the N-terminal domain called the gating loop (GL) and catalytic loop (CL). The GL in the two apo-forms exclude NADPH binding (Figure-2) through an intricate network of interactions, which progressively perished as they approached an NADPH-binding state. These changes were also accompanied by a change in orientation of linkers that connect the R-domains with carrier domains that carry the final product to its active site for product release. These observations allowed the proposition of a “Concerted loop movement” model in the present work. According to this model, R-domains only allow a fully mature product to be reductively released because conformations with no interactions prevent NADPH binding (Figure-3). Thus, the model provides yet another example of coordinated cross-talk between domains and modules of NRPS.

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 that causes 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 LipA and CbsA (N-terminal catalytic domain). LipA protein belongs to a new class of cell wall-degrading enzymes which has a unique mode of substrate recognition. CbsA (catalytic domain), belongs to the glycosyl hydrolase 6 (GH-6) family. Conversion of its aspartic acid at 131st position to alanine confers endoglucanase activity to the mutant protein. This altered enzymatic activity of D131A CbsA protein affects the virulence promoting activity of CbsA but does not affect the ability of the protein to induce rice innate immune responses. The C-terminal region of CbsA has a fibronectin type 3 (FN3) domain. A deletion mutant of FN3 domain was found to be deficient in virulence. Western blot analysis indicate that this deficiency is due to less secretion of the CbsA protein in Δ FN3 mutant when compared to the wild type. Currently we are trying to understand if FN3 domain has any role in the transport of CbsA protein. Interestingly, in the presence of FN domain the catalytic domain is biochemically inactive. Using X-ray crystallography and SAXS, we are trying to understand how the presence of FN3 domain affects the biochemical activity of catalytic domain of CbsA.



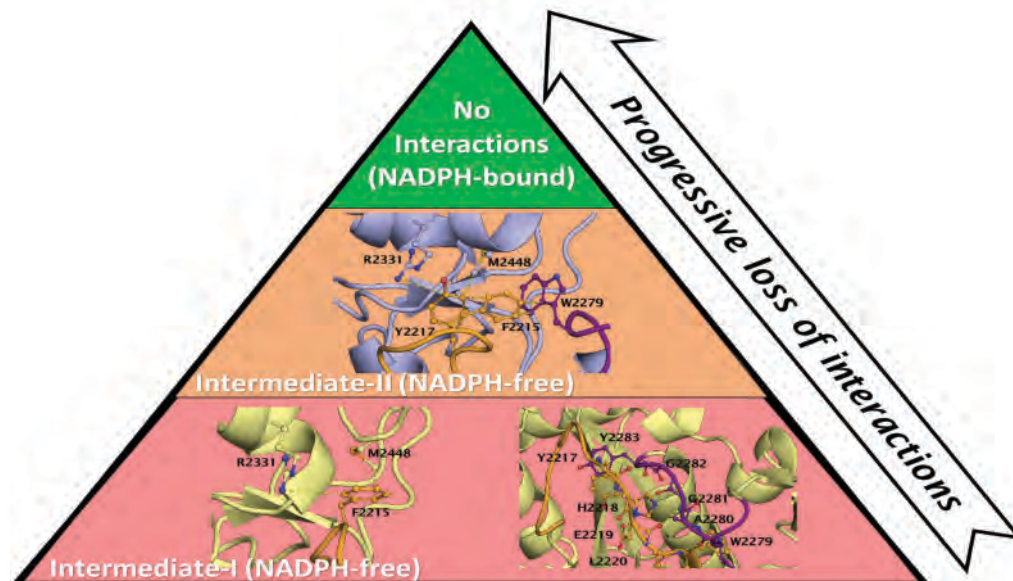


Fig.3: The concerted loop model depicted as a triangle with the base representing NADPH-free form with highest number of interactions between GL and CL depicted as cartoon in the section coloured light red. Sequential loss of some of these interactions to another NADPH-free form depicted as cartoon in the section coloured orange while the interaction-free GL and CL are depicted as cartoon in the section coloured blue.

In collaboration with Dr.Yogendra Sharma's group, we have established the double clamp motif (N/D-N/D-X₁-X₂-T/S-S) for binding Ca²⁺ in the $\beta\gamma$ -Crystallin superfamily of proteins. Recently we reported a member of $\beta\gamma$ -Crystallin superfamily, methallin which binds to transition metal ions with nano-molar affinity. Methallin is a protein that belongs to *Methanosaeta-thermophila*, an

archaea where members of $\beta\gamma$ -Crystallin superfamily are sparsely present with only three reported in archeal whole genome sequences. Methallin forms a trimer, with a single transition metal ion at the trimeric axis which is chelated by two histidine residues coming from each protomer (Figure-4). We are further exploring $\beta\gamma$ -Crystallins that bind transition metals.

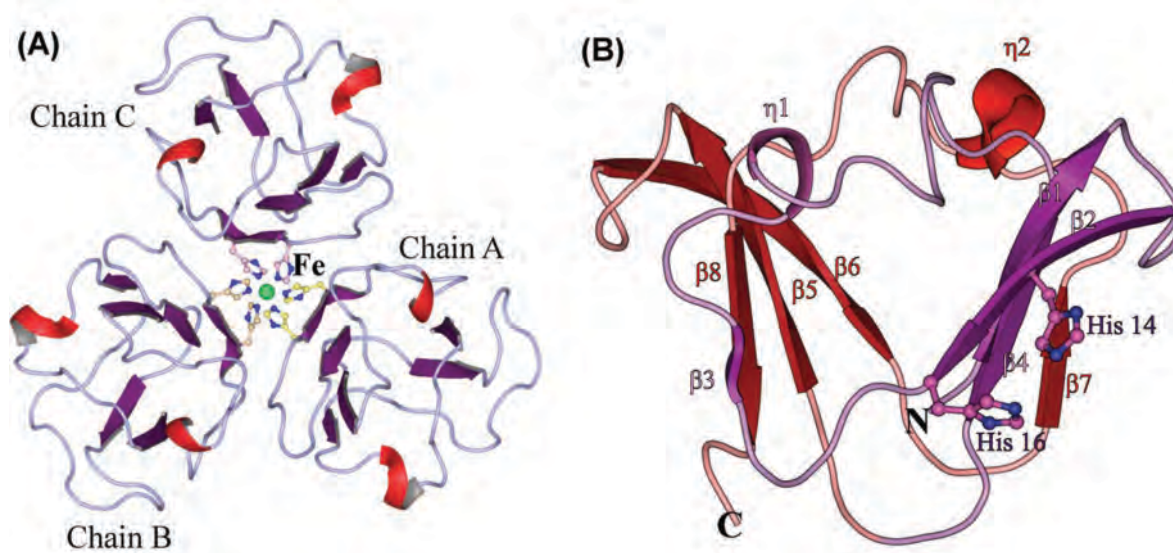


Fig.4: Crystal structure of $\beta\gamma$ -crystallin "Methallin" from methanoarchaea *M. thermophila*. (A) Cartoon representation of crystal structure of Methallin shows transition metal bound at the trimeric axis chelated by three histidine residues from each protomer. (B) A protomer of Methallin is comprised of two Greek key motifs (purple and red) with the β -strands forming a typical $\beta\gamma$ -crystallin domain. Two histidine residues (His14 and His16), which coordinate the metal ion, are located on the second β -strand.

Yogendra Sharma

Calcium Signaling via Calcium-binding Proteins



From left to right (top): Yogendra Sharma, Syed Sayeed Abdul, P Sai Uday Kiran, Radhika Khandelwal, R Phanindranath, Aditya A Jamkhindikar, Asmita Dhansing Pawar, Anand Kumar Sharma. (bottom): Bal Krishnan Mishra, Venu Sankeshi, Shanti Swaroop Srivastava, Rashmi Thapa, Amrutha HC

RESEARCH INTERESTS:

- $\beta\gamma$ -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 bacterial systems, we were instrumental in the discovery and establishment of a novel Ca^{2+} -binding protein superfamily, i.e., the $\beta\gamma$ -crystallins. Currently, we are deciphering the anti-diabetic efficacy of a β -cell enriched Ca^{2+} sensor protein, Secretagogin.

Selected recent publications

1. Kiran U, Regur P, Kreutz M, Sharma Y and Chakraborty A (2017) Intermotif communication induces hierarchical Ca^{2+} filling of Caldendrin. *Biochemistry* 56:2467-2476.
2. Khandelwal R, Sharma AK, Chadalawada S and Sharma Y (2017) Secretagogin is a redox-responsive Ca^{2+} sensor. *Biochemistry* 56:411-420.
3. Srivastava SS, Jamkhindikar A, Raman R, Jobby M K, Chadalawada S, Sankaranarayanan R and Sharma Y (2017) A transition metal-binding, trimeric $\beta\gamma$ -crystallin from methane producing thermophilic archaea *Methanosaeta thermophila*. *Biochemistry* 56:1299-1310 (featured on journal cover page).
4. Rajanikanth V, Sharma AK, Rajyalakshmi M, Chandra K, Chary KV and Sharma Y (2015) Liaison between Myristoylation and Cryptic EF-Hand Motif Confers Ca^{2+} Sensitivity to Neuronal Calcium Sensor-1. *Biochemistry* 54(4):1111-1122.
5. Srivastav SS, Mishra A, Krishnan B and Sharma Y (2014) Ca^{2+} -binding motif of $\beta\gamma$ -crystallins. *J. Biol. Chem.* 289(16):10958-10966. (Minireview).

Among the various types of Ca^{2+} -binding proteins, the best-studied have an EF-hand motif to coordinate the cation. However, 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 $\beta\gamma$ -crystallin superfamily. We have classified $\beta\gamma$ -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, $\beta\gamma$ -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 $\beta\gamma$ -crystallin. 'Methallin' from *Methanosaeta thermophila*, the largest producer of methane, does not have a 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 $\beta\gamma$ -crystallin led us to conclude that Methallin is a naturally designed trimer for high-affinity transition metal binding. While many $\beta\gamma$ -crystallins are shown to bind Ca^{2+} , and form homodimers and oligomers, a transition metal-binding, trimeric $\beta\gamma$ -crystallin is a new paradigm.

In another program under the broad interest of regulation of Ca^{2+} functions in eukaryotic systems, we have been studying the functions of 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 crucial 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 be indeed 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 SNPs affect 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 sensitive oligomerization and a higher affinity for Ca^{2+} in a reducing milieu and gains stabilization. The redox-responsive nature of SCGN was supported by its response to the DTT-induced stress in the MIN6 cells. Our report of redox sensitive dimerization finds significance in the recent report implicating SCGN dimerization in insulin secretion.

Imran Siddiqi

Plant Reproductive Biology



From left to right (back): S Prashanthi, Aparna Singh, Imran Siddiqi, V Subbiah, V Vijaybhaskar, Saurabh Pandey, Jayesh N Davda, VP Saradhi Attuluri.
(front): Dipesh K Singh, Aswan Nalli, Kaladhar B, Avinash, Frank Keith Max

RESEARCH INTERESTS:

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

Selected recent publications

1. Andreuzza S, Nishal B, Singh A and I Siddiqi (2015). The Chromatin Protein DUET/MMD1 Controls Expression of the Meiotic Gene TDM1 During Male Meiosis in Arabidopsis. *PLoS Genetics* 11(9):e1005396. doi: 10.1371/journal.pgen.1005396.
2. Qin Y, Zhao L, Skaggs M, Andreuzza S, Tsukamoto T, Panoli A, Wallace KN, Smith S, Siddiqi I, Yang Z, Yadegari R, and Palanivelu R (2014) ACTIN-RELATED PROTEIN6 regulates female meiosis by modulating meiotic gene expression in Arabidopsis. *Plant Cell* 26: 1612-1628.
3. Marimuthu MPA, Jolivet S, Ravi M, Pereira L, Davda, JN, Cromer L, Wang L, Nogue F, Chan SWL, Siddiqi I and R Mercier(2011). Synthetic clonal reproduction through seed. *Science*, 331:876.

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.

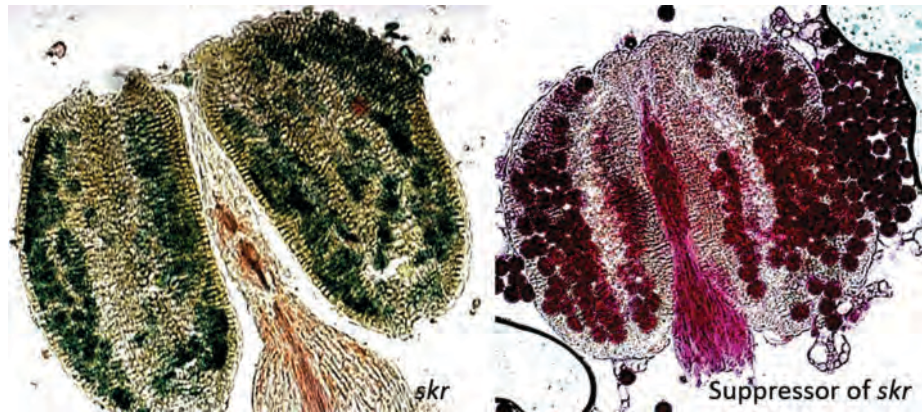


Fig.1: Suppressor of *skr* (M2 plant 127) showing restoration of viable pollen

Dr. Imran Siddiqi's 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 breeding to meet the challenges facing agriculture with respect to increasing food production for a growing world population. The group has identified and analyzed several genes that control different aspects of meiotic chromosome organization. Some of these genes are unique to plants whereas others show conservation to meiotic genes in other eukaryotes.

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 apomixis 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* 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 a suppressor (*ssk*) which restores male fertility and seed set in a *skr* mutant background. Characterization of *ssk* is in progress.

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 postmeiotic 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 postmeiotic 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.

Puran Singh Sijwali

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



From left to right: Nandita T, S Thanumalayan, Manish Bhattacharjee, Deepak Kumar, Neeradi Dinesh, Zeba Rizvi, Puran Singh Sijwali, Navin Adhikari, Amit Nagwani, Divya Das and Renu Sudhakar

RESEARCH INTERESTS:

- Ubiquitin proteasome system
- Autophagy
- Lysosomal Proteases
- Antimalarial targets
- Malaria vaccine development

Selected recent publications

1. Ashok Yadav P, Pavan Kumar C, Siva B, Suresh Babu K, Allanki AD, Sijwali PS, Jain N, Veerabhadra Rao A. (2017) Synthesis and evaluation of anti-plasmodial and cytotoxic activities of epoxy-azadiradione derivatives. *Eur J Med Chem*. 134:242-257.
2. Al-Nihmi FM, Kolli SK, Reddy SR, Mastan BS, Togiri J, Maruthi M, Gupta R, Sijwali PS, Mishra S and Kumar KA (2017) A Novel and conserved *Plasmodium* sporozoite membrane protein SPELD is required for maturation of exo-erythrocytic forms. *Sci Rep*. 7: 40407.
3. Klionsky et al. (2016) Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy* 12(1):1-222. (REVIEW).
4. Balabadra S, Kotni M, Manga V, Allanki AD, Prasad R and Sijwali PS (2016) Synthesis and evaluation of naphthyl bearing 1,2,3-triazole analogs as antiplasmodial agents, cytotoxicity and docking studies. *BioOrg Med Chem*. 2017;25(1):221-232.

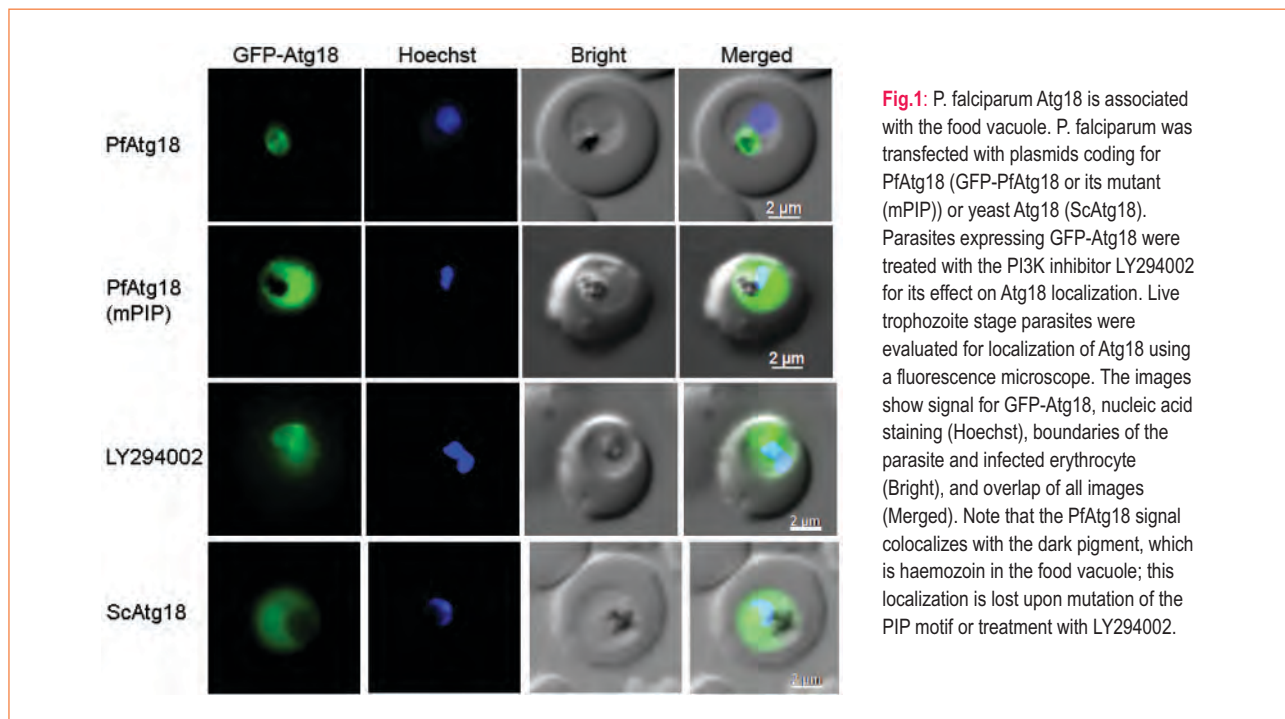
Our group has showed that Malaria parasites have a limited autophagy repertoire with atypical features and indispensable role

We are studying the proteolytic systems of malaria pathogen *Plasmodium* to determine their roles in parasite biology and disease pathogenesis. Multi-stage development of malaria parasites in different intracellular and extracellular environments has been a major challenge to design/develop therapies for disease control/elimination. A major research interest of the lab is to identify proteases 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 is a lysosome-dependent process that delivers a variety of cellular contents, including organelles, to the lysosome, for degradation. However, autophagy 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 revealed a limited autophagy repertoire in malaria parasites, which comprises only 15 proteins as opposed to ~40 proteins in mammalian cells and yeast. It includes the canonical autophagy marker Atg8 and the enzymes required for its conjugation to the membranes. Immunolocalization studies showed that Atg8 is expressed and associated with punctate structures in all the major stages. It is exclusively found in the pellet fraction as an integral membrane protein, which is in contrast to the yeast or mammalian Atg8 that is distributed in both cytosolic and membrane fractions, suggesting that a constitutive autophagy is operative in malaria parasites. Starvation is a typical autophagy inducer that enables cells to survive the starvation phase by recycling dispensable cellular contents, which accompanies with increased Atg8 expression level and the number of Atg8-associated puncta. However, starvation decreased PfAtg8 level. Neither the Atg8-associated puncta nor the Atg8 expression level was noticeably altered upon treatment of parasites with routinely used autophagy inhibitors. Prolonged inhibition of the major food vacuole protease activity did not cause accumulation of the Atg8-associated puncta in the food vacuole, suggesting that autophagy is primarily not meant for degradative function in malaria parasites.

To investigate how Atg8-associated structures are formed, we are studying Atg18 that has been shown



to have a role in nucleation and assembly of the phagophore, a cup-like Atg-8 labelled structure. Immunolocalization experiments showed that Atg18 is expressed in all erythrocytic stages of *P. falciparum*, with exclusive localization to the food vacuole, the parasite lysosome wherein bulk degradation of haemoglobin occurs (Figure 1). Mutations in a putative phosphatidylinositol-binding motif of PfAtg18 completely altered its localization from the food vacuole to cytosol (Figure 1). Similar effect was also observed upon treatment with the PIP3K inhibitor LY294002 (Figure 1), indicating that binding with the phosphatidylinositol is essential for Atg18 localization to the food vacuole. However, yeast Atg18, which shares the same motif and has been shown to bind to phosphatidylinositol, did not localize to the food vacuole (Figure 1), suggesting that the presence of phosphatidylinositol-binding motif alone is not sufficient for recruitment to the food vacuole. Several antimalarials and inhibitors were assessed for their effects on Atg18. Interestingly, some of the

antimalarials showed a noticeable effect on Atg18 localization and expression, suggesting that Atg18 is another target by which these antimalarials kill malaria parasites.

Phagophore is expanded into the autophagosome by Atg8 and Atg12-Atg5-Atg16 conjugation systems. The Atg12-Atg5-Atg16 conjugation system appears to be incomplete in malaria parasites, as Atg10 and Atg16 homologs are absent. Nonetheless, results from the gene knockout/knock-down experiments indicate that Atg12-Atg5 conjugation system is essential for parasite development. This raises interesting questions about the role and mechanisms by which the Plasmodium Atg12-Atg5 conjugation system works. Collectively, our results reveal several atypical features of the autophagy system in malaria parasites, and also demonstrate its indispensability for parasite development. Studies are underway to dissect the autophagy repertoire and determine its roles in parasite development.

Shashi Singh

Adult Stem Cells from Various Sources and Tissue Engineering



Shashi Singh

RESEARCH INTERESTS:

- Use of adult stem cells for lineage derivation by alteration of niche, small molecules etc. Tissue engineering using modified niche, natural scaffolds etc.

Our lab is working on Adult stem cells and tissue engineering. Stem cells obtained from medical waste like placenta, Wharton's jelly, cord blood, bone marrow and adipose lipo-aspirate are expanded and differentiated into different lineages. Tissue engineering involves creation of organoids or tissue constructs with cells, associated matrices and few factors in order to produce a functional structure. For regenerative medicine, stem cells can also be injected directly at the site of injury or intravenously for injuries like spinal cord, bone repair, cardiac, etc., and the entire tissue can be reconstructed by tissue engineering such as cornea, liver, pancreas.

Selected recent publications

1. Begum G, Reddy T N, Kumar K P, Dhevendar K, Shashi Singh, Amarnath M, Misra S, Rangari VK and Rohit K R (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 (34):22056–22063 doi: 10.1021/acsami.6b07177.
2. Bondarenko O M, Heinlaan M, Sihtmäe M, Ivask A, Kurvet I, Joonas E, Jemec A, Mannerström M, Heinonen T, Rekulapelly R, Singh S, Zou J, Pyykkö I, Drobne D, Kahru A. (2016) Multilaboratory evaluation of 15 bioassays for (eco) toxicity screening and hazard ranking of engineered nanomaterials: FP7 project NANOVALID. *Nanotoxicology* 10(9):1229-42. doi: 10.1080/17435390.2016.1196251. Epub 2016 Jun 28.
3. Kos M, Kahru A, Drobne D, Shashi Singh, Kalčíková G, Kühnel D, Rohit R, Gotvajn AZ, and Jemec A (2016) A case study to optimise and validate the brine shrimp *Artemiafranciscana* immobilisation assay with silver nanoparticles: The role of harmonization. *Envir. Pollution* 213: 173-183.
4. Jemec A, Kahru A, Potthof A, Drobne D, Heinlaan M, Böhme S, Geppert M, Novak S, Schirmer k, Rohit R, Shashi Singh, Aruoja V, Sihtmäe M, Juganson K, AKäkinen, and Kühnel D. (2016) An interlaboratory comparison of nano silver characterisation and hazard identification: Harmonising techniques for high quality data *Environment International* 87: 20–32.
5. Snehal R and Shashi Singh (2016) Calcium-phosphate nanobioceramics. *Lett in Applied Nano BioScience* 5: 389-396.

Differentiation of MSC and their compatibility with Scaffolds

We are using some of the matrix proteins to create matrices for cell growth and differentiation. Collagen Type I extracted from rat tails is being used along with fibronectin, laminin and proteoglycans or plant based glycan rich exudates (gums) for making electro-spun fibers that are collected on coverslips. Various cells like Mesenchymal Stem Cells (MSC) or differentiated cells are plated and their growth pattern is followed over a period. The cells are finally checked for their differentiation state.

3D scaffolds of matrix proteins with plant based gums in form of hydrogels, have been created for matrix driven differentiation of MSC. A series of constructs with different gums and protein ratios are screened for various cell types. Our screen reveals differential differentiation of cells in various compositions.

Natural scaffolds: We have successfully obtained organ scaffold by decellularizing for Pancreas and Kidney. The pancreatic scaffold was recellularized using mesenchymal stem cells. Efficacy of the recellularized structures was tested by implanting in streptozotocin induced diabetic mice. The mice started showing gluco-regulation by day 25 and the structure of the implanted organ resembles a normal pancreas with acini and fully developed islets. The immunolocalization of exocrine and endocrine markers showed the positive reaction in respective segments. The vascularization of the grafted pancreas also appeared normal. The empty scaffold without recellularization always resulted in graft rejection. The recellularization was done using murine cells or Human MSC and both the structures

were well tolerated by the mice up to 40 days. Analysis of mouse serum of implanted vs. sham operated mice did not show any difference in cytokines up to 30 days. The development pathways involved in the course of transition of MSCs into the pancreatic cell types is being worked out in this process.

Differentiation/Dedifferentiation using small molecules

We are interested in screening of small molecules for achieving reprogramming of cells. The idea is to look for molecules targeting signaling pathways leading to an altered state of cells. We are aiming for both dedifferentiation of cell into iPS and differentiation into beta cells. We ran parallel screens for Oct 4 and Nanog, and also other signaling pathways reporters, Wnt, Notch and the Hedgehog pathway. We have screened about 135 molecules and have shortlisted 10 for final screening after validation. Since these molecules were inducing Oct4 and Nanog, our next screen was to use them with the chemi-inducers for iPS and we shortlisted two molecules. We are further working on these molecules to induce human cells into iPS.

In another screen we are targeting antidiabetic molecules. We have designed about 7 tests for antidiabetic properties include effects for type I and type II diabetes. In this category we have about 67 molecules that were screened for the 7 tests. These tests will also pick up molecules that can be used for induction of cells to beta cell types and among the set of 67 molecules we have shortlisted 3 for direct reprogramming, these molecules are being further tested for validation.

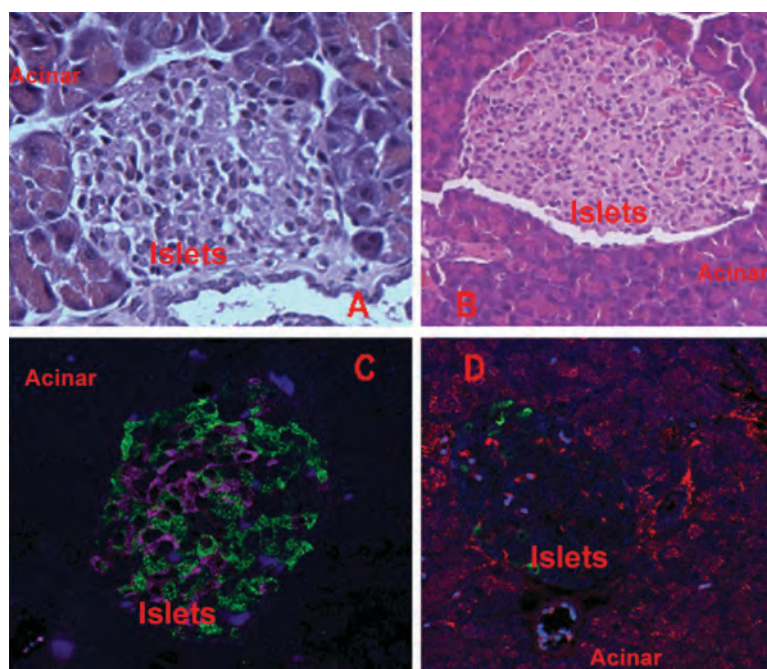


Fig.1: Natural scaffolds obtained by decellularization of Pancreas repopulated by MSC obtained from mice (A), Human MSC (B) and stained with markers for islets and Acinar cells show re-generation of the organ. Islets cells stained for beta cell using insulin antibody (green) and Delta cells using somatostatin antibody (Red) in Fig C; Fig D shows beta cells stained for neurogenin 3 (green) and Acinar cell stained red for Carboxypeptidase.

Sadanand D Sontakke

Reproductive Biotechnologies for Wildlife Conservation



Sadanand D Sontakke

RESEARCH INTERESTS:

- Assisted Reproductive Technologies
- Molecular regulation of ovarian function
- Effect of Inbreeding on reproductive function

My research focuses on understanding molecular regulation and control of ovarian function, and to develop assisted reproductive technologies to support conservation and planned breeding of endangered wildlife.

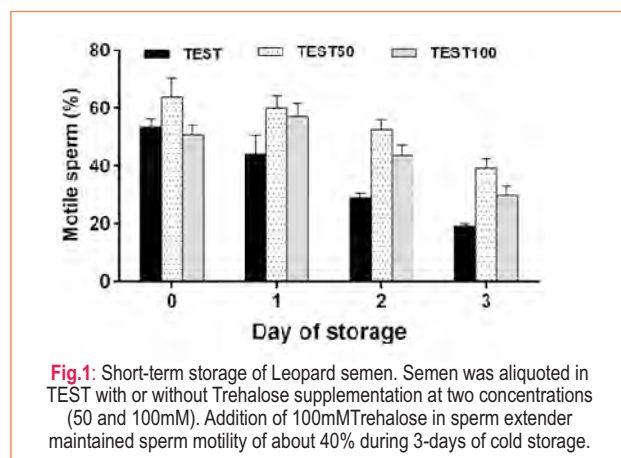
Selected recent publications

1. Sontakke SD, Umapathy G and Shivaji S (2009) Yohimbine antagonizes the anesthetic effects of ketamine-xylazine in captive Indian wild felids (lions, tigers and leopards) *Veterinary Anesthesia and Analgesia* 36 :34- 41.
2. Donadeu FX, Sontakke SD and Jason I (2016) MicroRNA indicators of follicular steroidogenesis *Reproduction, Fertility and Development* 29(5)906-912.
3. Bushra T M, Sontakke SD, Ioannidis J, Duncan WC and Donadeu FX (2017) The adequate corpus luteum: miR-96 promotes luteal cell survival and progesterone production. *Journal of Clinical Endocrinology and Metabolism* doi: 10.1210/jc.2017-0025.
4. Sontakke SD, and Umapathy G (2017) A manual on Chemical immobilization of wild animals. CSIR-CCMB and Central Zoo Authority, New Delhi publication (in press).

Studies on semen preservation of endangered Indian Leopard for effective captive breeding

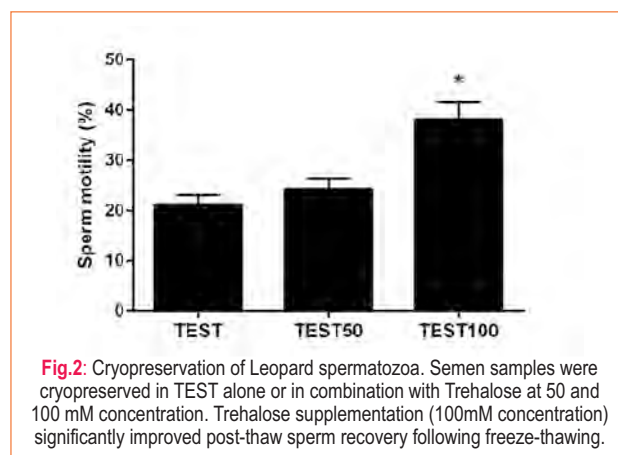
Assisted reproduction and semen banking go hand-in-hand for effective captive breeding and genetic management of stagnated captive population. Undoubtedly, semen cryopreservation is a valuable tool in wildlife conservation because it facilitates the dispersion of the existing gene pool without the risks associated with transportation of valuable sires between zoos. Although, semen cryopreservation has been very effective in various ungulates, it had a limited success in wild felids because spermatozoa of felids are sensitive to cold shock due to high content of polyunsaturated fatty acids in sperm membrane. The maximum post-thaw sperm recovery of cryopreserved semen reported so far in any wild felid is < 40%. In such instances, fertilizing efficiency of frozen sperm remains questionable for a successful artificial insemination. So ideally, in addition to the long-term semen cryopreservation, development of short-term preservation method would be useful in exchange of gene pool between zoos and even from the wild. The present study aimed to develop better ways of semen preservation, viz. cold storage (at refrigerator) and also an efficient cryo preservation protocol (in liquid nitrogen) of leopard spermatozoa in order to facilitate conservation breeding of wild felids in captivity.

Short-term semen storage: Semen samples were collected by electro-ejaculation procedure (Jayaprakash *et al.* 2001) under general anaesthesia (Sontakke *et al.* 2009), from 3 leopards housed at the LaCONES Animal Holding Facility. Samples were diluted with Tes-*n*-Tris-yolk buffer (TEST) with or without supplementation of trehalose at two different concentrations (final conc. 50 and 100 mM) and were stored in the refrigerator (at ~ 5°C) for 3 days. Sperm variables such as motility and acrosomal integrity declined over a period of storage. Supplementation of trehalose at both concentrations significantly improved these sperm parameters, and the maximum



sperm recovery was obtained in 50 mM trehalose-treated samples than those in 100 mM trehalose or TEST alone. Sperm motility up to 50% was maintained for up to 3 days of cold storage (Figure 1). Such cold-stored semen could be easily shipped by road or air within the country from one zoo to another in a cold container for use in a short period of time (up to 3 days).

Cryopreservation of semen: Previous study on cryopreservation of leopard semen in our laboratory resulted in only 30% of post-thaw sperm recovery (Jayaprakash *et al.* 2001). Therefore, the present study was carried out to improve the cryopreservation efficiency of leopard semen with the addition of Trehalose. Semen samples were subjected to slow freezing in TEST cryoextender with two concentrations of trehalose (50 and 100mM) or control TEST extender alone. It was found that trehalose supplementation at 100mM concentration but not 50 mM significantly improved post-thaw sperm recovery of leopard spermatozoa yielding 40-45% of viable motile sperm post-thawing as compared to without trehalose. Further, the acrosomal integrity of spermatozoa was comparatively better in trehalose supplemented samples.



In conclusion, our studies suggest that leopard semen (as a model for highly endangered felids) could be successfully preserved at refrigeration for easy shipment from one zoo to another within 3 days. Further, supplementation of Trehalose sugar in cryoextender improved post-thaw recovery of leopard sperm motility. The fertilizing efficiency of the cold-stored and cryopreserved spermatozoa needs to be addressed by carrying out artificial insemination trials. These studies would help for establishment of genome banking and conservation breeding of endangered wild felids in India.

Ramesh V Sonti

Plant-Pathogen Interactions



From left to right (front row): Hitendra Kumar Patel, B. Kranthi, Sohini Deb, Rajkanwar Nathawat, Neha Kachewar, Shailaja Kanumuri, Sandhya, Hota Shiva Jyothi, (middle row): TayiLavanya, Ramesh V. Sonti, Chandan Kumar, Shakuntala Pillai, Vishnu Narayanan, Raju Madanala, Sridivya Vungarala, (back row): Roshan MV, Bipin Kumar, Gokulan CG, Kamal Kumar Malukani

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

1. Sujatha S, Alok P, Vishnupriya MR, Hitendra K Patel and R V Sonti (2011) The ColRS system of *Xanthomonasoryzaepv. oryzae* is required for virulence and growth in iron-limiting conditions. *Molecular Plant Pathology* 13: 690-703.
2. Dipanwita S, Mahesh K G, Hitendra K Patel, Ashish R and R V Sonti. (2013). Cell wall degrading enzyme induced rice innate immune responses are suppressed by the Type 3 secretion system effectors XopN, XopQ, XopX and XopZ of *Xanthomonasoryzaepv. oryzae*. *PLoS ONE*, 8(9): e75867.
3. Mahesh K G, Dipanwita S, Rajkanwar N, AsfarulHaque, Sankaranarayanan R and R V Sonti (2015). Mutations in the predicted active site of *Xanthomonasoryzaepv. oryzae*XopQ differentially affect virulence, suppression of host innate immunity and induction of HR in a non-host plant. *Molecular Plant-Microbe Interactions* 28: 195-206.
4. LavanyaT, Roshan M V, Hitendra K Patel and R V Sonti (2016). Action of multiple cell wall degrading enzymes is required for elicitation of innate immune responses during *Xanthomonasoryzaepv. oryzae* infection in rice.' *Molecular Plant-Microbe Interactions*. <http://dx.doi.org/10.1094/MPMI-02-16-0039-R>.
5. Anil M G, Bipin K K, Madhavi M B, Ramesh P, Sridivya V, Hitendra K Patel and Ramesh V Sonti. (2017). Rice leaf transcriptional profiling suggests a functional interplay between *Xanthomonas-oryzaepv. oryzae* lipopolysaccharide and extracellular polysaccharide in modulation of defense responses during infection. *Molecular Plant-Microbe Interactions*. 30 (1): 16-27. <http://dx.doi.org/10.1094/MPMI-08-16-0157-R>.

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* (Xoo) and its 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; CIsA) 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. 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.

What are the T3S secreted proteins of Xoo that suppress rice innate immune responses? We have cloned into Agrobacterial T-DNA vectors, the genes for 16 different T3S secreted proteins of Xoo. Our results obtained using Agrobacterium-mediated transient transfection assays indicate that 4 out of these 16 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. Genetic analysis suggests that there is a redundancy with regard to the functioning of these proteins in suppression of host innate immune responses and that even one of these four effectors can suppress rice innate immunity. The XopQ protein is homologous to nucleoside hydrolases in bacteria and protozoa. However, purified XopQ protein does not have any activity against standard nucleoside substrates but does exhibit a weak ribose hydrolase activity against the chromogenic substrate 4-nitrophenyl β -D-ribofuranoside. Mutants of XopQ that are defective in biochemical activity have been identified. These mutations affect the ability of XopQ protein to promote virulence of Xoo on rice but do not affect ability to suppress innate immunity. The XopQ homolog of a xanthomonad pathogen that infects

plants such as tomato and tobacco has been shown recently to suppress host defense responses by interaction with plant 14-3-3 proteins. We have created a XopQ variant with mutation in the 14-3-3 binding motif and this mutant is defective in the ability to suppress rice innate immunity. Our current hypothesis is that Xoo XopQ suppresses rice innate immune responses by interacting with host 14-3-3 (signaling) proteins and that XopQ has an additional role in promoting virulence that is independent of the ability to suppress innate immunity. We postulate that the biochemical activity of XopQ is involved in this additional role.

In our earlier studies, the major cellulases and xylanases secreted by Xoo have been identified and their role in virulence was determined. In one of our recent studies, we have further identified the pectin degrading enzymes of Xoo and assessed their role in virulence. Bioinformatics analysis indicated the presence of four pectin homogalacturonan (HG) degrading genes in the genome of Xoo. The four HG degrading genes include one polygalacturonase (*pglA*), one pectin methyl esterase (*pmt*) and two pectate lyases (*pel* and *pelL*). There was no difference in the expression of *pglA*, *pmt* and *pel* genes by laboratory wild type Xoo grown in nutrient rich PS medium and in plant mimic XOM2 medium whereas the expression of *pelL* gene was induced in XOM2 medium as indicated by qRT-PCR experiments. Gene disruption mutations were generated in each of these four genes. The polygalacturonase mutant *pglA* was completely deficient in degrading the substrate Na-polygalacturonic acid (PGA), while pectin methyl esterase mutant *pmt* and mutants of pectate lyases *pel* and *pelL* were as efficient as wild type Xoo in cleaving PGA, clearly indicating that PglA is the major pectin degrading enzyme produced by Xoo. Pectin methyl esterase Pmt is the pectin de-esterifying enzyme secreted by Xoo as evident from the enzymatic activity assay performed using pectin as the substrate. Mutations in these pectin degrading genes *pglA*, *pmt*, *pel* and *pelL* have minimal effects in virulence on rice as compared to wild type Xoo. This suggests that, as compared to cellulases and xylanases, this class of CWDEs may have no major role in the pathogenicity of Xoo.

Prior treatment of rice leaves with purified CWDEs such as LipA can confer enhanced resistance against subsequent Xoo infection. In order to understand CWDE-induced rice defense responses, microarray analysis was performed 12 h after enzyme treatment of rice leaves. The analysis revealed that 42 out of a

total of 267 differentially expressed genes encode transcription factors (TFs). Transient overexpression of one of the highly expressed TFs, namely OsRERJ1 (a helix loop helix TF), in rice has been shown to induce immune responses. Rice lines with mutations in OsRERJ1 show defects in their ability to induce immune responses following treatment with CWDEs suggesting that OsRERJ1 plays an important role in CWDE induced defense response. Furthermore, inducible heterologous over expression of OsRERJ1 in independent Arabidopsis lines has also been shown to induce callose deposition and enhanced tolerance to the bacterial pathogen, *Pseudomonas syringae*. This suggests that the immune response pathways modulated by OsRERJ1 is broadly conserved across species.

In collaboration with the Indian Institute of Rice Research (formerly Directorate of Rice Research), an ethyl methane sulfonate mutagenized population has been developed in the genetic background of the elite rice variety, Samba Mahsuri. The mutagenized population has been screened for resistance/

tolerance to the yellow stem borer insect pest, sheath blight fungal disease and to Xoo. Several advance generation mutagenized lines were found to be tolerant to the rice insect pest-yellow stem borer. Several of these lines are found to have a promising level of tolerance and will be tested in farmers' fields during Kharif 2017. Several mutant lines that are tolerant to the serious fungal disease, sheath blight, and others that are tolerant to Xoo have been identified and are being characterized. In addition to these lines which exhibit biotic stress tolerance, many stabilized mutant lines have also been identified which are high yielding, exhibit complete panicle emergence (CPE; which also leads to high yield), mature early, have strong culm/stem characteristic (has greater capacity to resist wind damage), etc. Selected entries including strong culm and CPE lines have been crossed with parent Samba Mahsuri and F₂ population have been phenotyped. F₂ mutant bulks for strong culm and CPE were sequenced and MutMap is being done to map the mutations in strong culm and CPE mutant rice lines.

Ghanshyam Swarup

Molecular Mechanisms of Neuro-degeneration caused by Mutations in Optineurin



From left to right : Zuberwasim Sayyad, Shivranjani C Moharir, Rajashree Ramaswamy, Ghansyam Swarup, Akhouri Kishore Raghawan, R Gopalakrishna

RESEARCH INTERESTS:

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

- Optineurin promotes autophagosome formation by potentiating LC3-II production and maturation of phagophore into autophagosome.
- Glaucoma-associated mutants of optineurin are defective in autophagy, vesicle trafficking and signalling.
- A disease-associated mutant of NLRC4, an innate immune receptor, constitutively activates caspase-8, in addition to activating caspase-1.

Selected recent publications

1. Raghawan A K, Sripada A, Gopinath G, Pushpanjali P, Kumar Y, Radha V and 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. *J Biol Chem*. 292(4):1218-1230.
2. Sirohi K and Swarup G (2016) Defects in autophagy caused by glaucoma-associated mutations in optineurin. *Exp Eye Res*. 144, 54-63.
3. Sirohi K, Chalasani ML, Sudhakar C, Kumari A, Radha V and Swarup G (2013) M98K-OPTN induces transferrin receptor degradation and RAB12-mediated autophagic death in retinal ganglion cells. *Autophagy* 94, 510-527.
4. Vaibhava V, Nagabhushana A, Chalasani ML, Sudhakar C, Kumari A and Swarup G (2012). Optineurin mediates a negative regulation of Rab8 by the GTPase activating protein TBC1D17. *J. Cell Science*, 125: 5026-5039.
5. Nagabhushana A, Chalasani ML, Jain N, Radha V, Rangaraj N, Balasubramanian D and Swarup G (2010). Regulation of endocytic trafficking of transferrin receptor by optineurin and its impairment by a glaucoma-associated mutant. *BMC Cell Biol*. 11: 4.

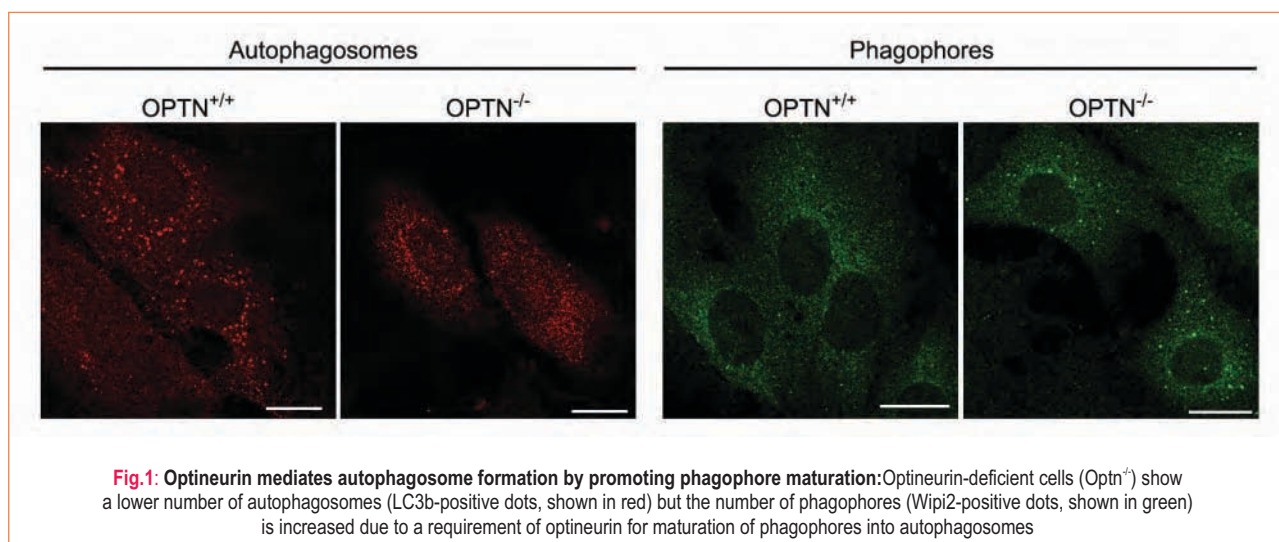
Molecular mechanisms of neurodegeneration caused by mutations in optineurin

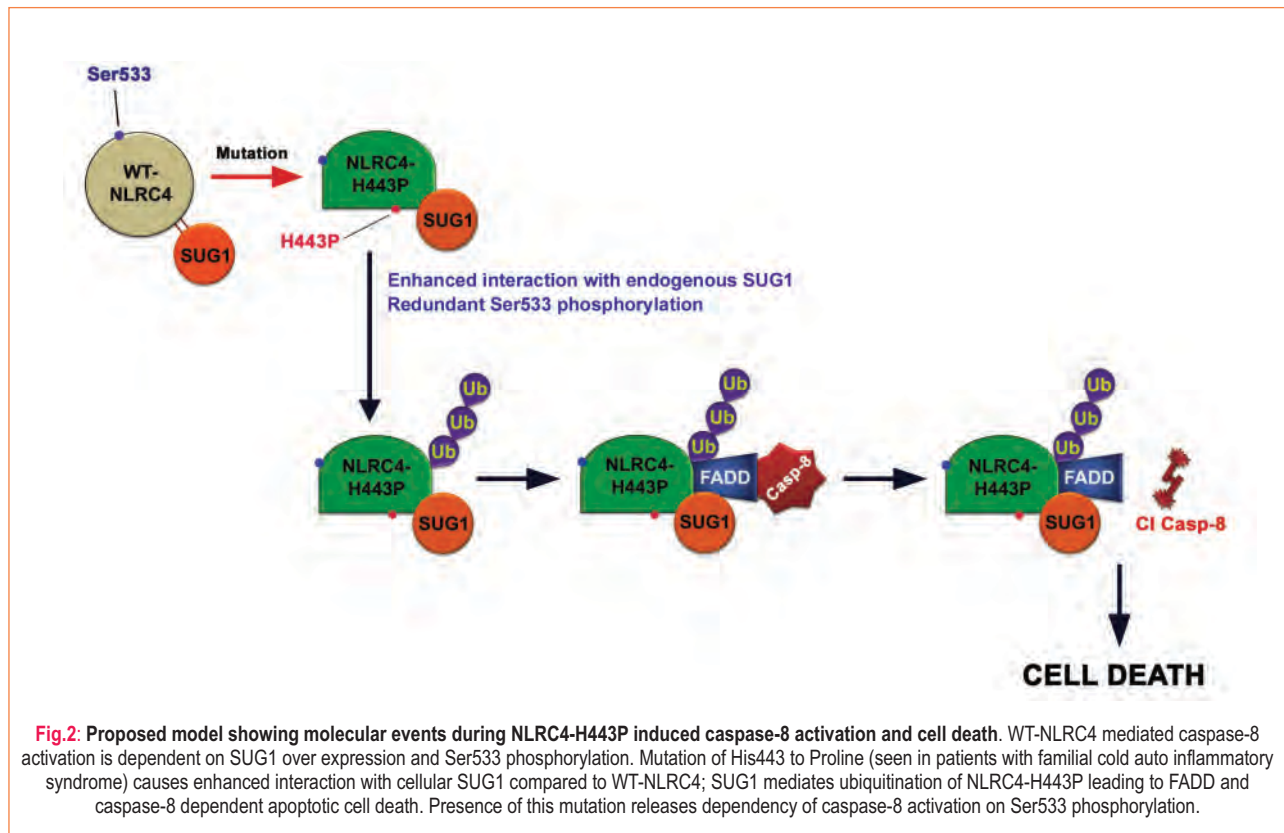
The central theme of our group's research is to understand the function of the protein optineurin and to study the functional defects caused by disease-associated mutations in optineurin. Mutations in the coding region of the gene *OPTN*, which codes for the protein optineurin, cause glaucoma and ALS (amyotrophic lateral sclerosis), both of which are neurodegenerative diseases. In glaucoma, the loss of vision is irreversible, which occurs due to progressive degeneration of retinal ganglion cells 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 retinal ganglion cells (RGCs) but some other mutants did not. This suggested that the E50K mutant causes glaucoma possibly by directly inducing death of RGCs. This is due to impaired transferrin receptor function 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 cell death.

The M98K polymorphism is associated with glaucoma in certain ethnic groups. Like the E50K mutant, M98K-OPTN induces cell death selectively in RGCs but not in other cells tested. However, unlike in E50K-induced cell death, M98K-induced cell death is not inhibited by antioxidants or antiapoptotic protein Bcl2. Expression of M98K induces autophagy leading to transferrin receptor degradation and cell death. Thus an appropriate level of autophagy is essential for survival of RGCs because enhanced autophagy, as seen with M98K-OPTN, or a block in autophagy, as shown by 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 young mice did not show any abnormality. The older mice are being examined for various functional and cellular/molecular alterations.

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 cells, which express several markers of RGCs 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. 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.





Mechanisms of autophagy and intracellular membrane vesicle trafficking

We have explored the role of optineurin in autophagosome formation. During autophagy, signaling events lead to the formation of a cup-shaped structure called the phagophore that matures into the autophagosome. Fibroblasts from the 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.

Rab GTPases control almost all the steps involved in trafficking of membrane vesicles. We have proposed a novel mechanism of regulating a Rab GTPase by an effector protein (optineurin) that acts as an adaptor to bring together a Rab (Rab8) and its GTPase activating protein (TBC1D17). The E50K mutant causes enhanced inhibition of Rab8 by TBC1D17

resulting in defective endocytic recycling of transferrin receptor.

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 (Ipad), a NLR family member, activates caspase-1 in response to intracellular pathogens to induce interleukin production. 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 known. 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.

Raghuhand R Tirumalai

Dissecting the Molecular Basis of *Mycobacterium tuberculosis* Pathogenesis



From left to right : Ravi Prasad Mukku, Mamata Savanagouder, Veena Chaitanya, Raghuhand Tirumalai, Vimal Kishore, Gopinath Viswanathan, R Gopalakrishna

RESEARCH INTERESTS:

- Host-pathogen interactions during *M.tb* infection
- Identification of mycobacterial virulence determinants
- Molecular correlates of *M.tb* latency
- 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

1. Viswanathan G, Yadav S and Raghuhand T R (2017) Identification of mycobacterial genes involved in β -lactam sensitivity - Implications in treatment of tuberculosis with β -lactam containing regimens. *Antimicrobial Agents and Chemotherapy* doi: 10.1128/AAC.00425-17.
2. Viswanathan G, Yadav S, Joshi S V, and Raghuhand T R (2017) Insights into the function of FhaA, a cell-division associated protein in mycobacteria. *FEMS Microbiology Letters* 364 (2): fnw294.
3. Yeruva V C, Savanagouder M, Khandelwal R, Kulkarni A M, Sharma Y and Raghuhand TR (2016) The *Mycobacterium tuberculosis* desaturase DesA1 (Rv0824c) is a Ca^{2+} binding protein. *Biochem Biophys Res Com* 480(1):29-35.
4. Yeruva VC, Kulkarni A M, Khandelwal R, Sharma,Y and Raghuhand T R (2016). The PE_PGRS proteins of *Mycobacterium tuberculosis* are Ca^{2+} binding mediators of host-pathogen interaction. *Biochemistry* 55(33):4675-87.
5. Sharma SGS, Kishore V and Raghuhand T R (2016) Identification and functional annotation of mycobacterial septum formation genes using cell division mutants of *Escherichia coli*. *Res Microbiol*, 167(2):142-8.

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 development of specific immunity often results in 'latent' infection but not eradication of the organism, leading to the possibility of reactivation tuberculosis occurring years after the initial exposure. The identification and characterisation of bacillary factors involved in evasion, and their interplay with host defence 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 discerning the molecular basis of latency, characterising the events at the host-pathogen interface, the identification of bacillary virulence factors and identifying novel antibiotic resistance mechanisms.

The *Mycobacterium tuberculosis* desaturase DesA1 (Rv0824c) is a Ca²⁺ binding protein

The hallmark feature of *M.tb* is its complex lipid rich cell wall comprised primarily of mycolic acids - long chain fatty acids that play a key role in structural stability and permeability of the cell wall. In addition, they are involved in inhibiting phagosome-lysosome fusion and aid in granuloma formation during the pathogenic process. *M.tb* DesA1 is an essential acyl-acyl carrier protein desaturase predicted to catalyze the introduction of position specific double bonds during the biosynthesis of mycolic acids. This protein is one among three annotated desaturases (DesA1-3) in the *M.tb* genome but is unique in containing a $\beta\gamma$ -crystallin Greek key signature motif, a well-characterized fold known to mediate Ca²⁺ binding in both prokaryotic and eukaryotic organisms. Using Isothermal Titration Calorimetry and ⁴⁵CaCl₂ overlay, we demonstrated that Ca²⁺ binds to DesA1 (Figure 1). Spectroscopic measurements suggested that this binding induces changes in protein conformation but does not lead to significant alterations in the secondary structure of the protein, a feature common to several $\beta\gamma$ -crystallins. An *M. smegmatis* strain over-expressing *M.tb desA1* showed a Ca²⁺ dependent variation in surface phenotype, revealing a functional role for Ca²⁺ in DesA1 activity. This study represents the first identification of a Ca²⁺ binding $\beta\gamma$ -crystallin in *M.tb*, emphasizing the implicit role of Ca²⁺

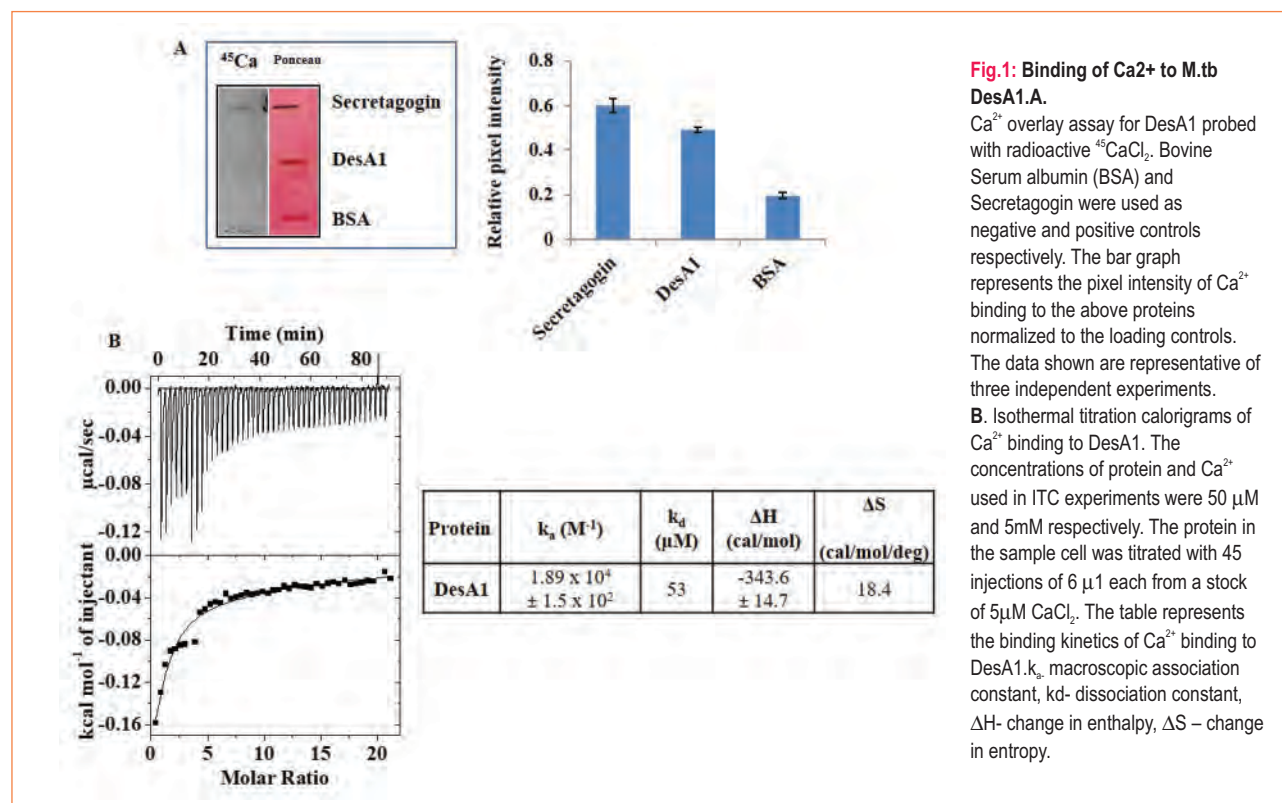
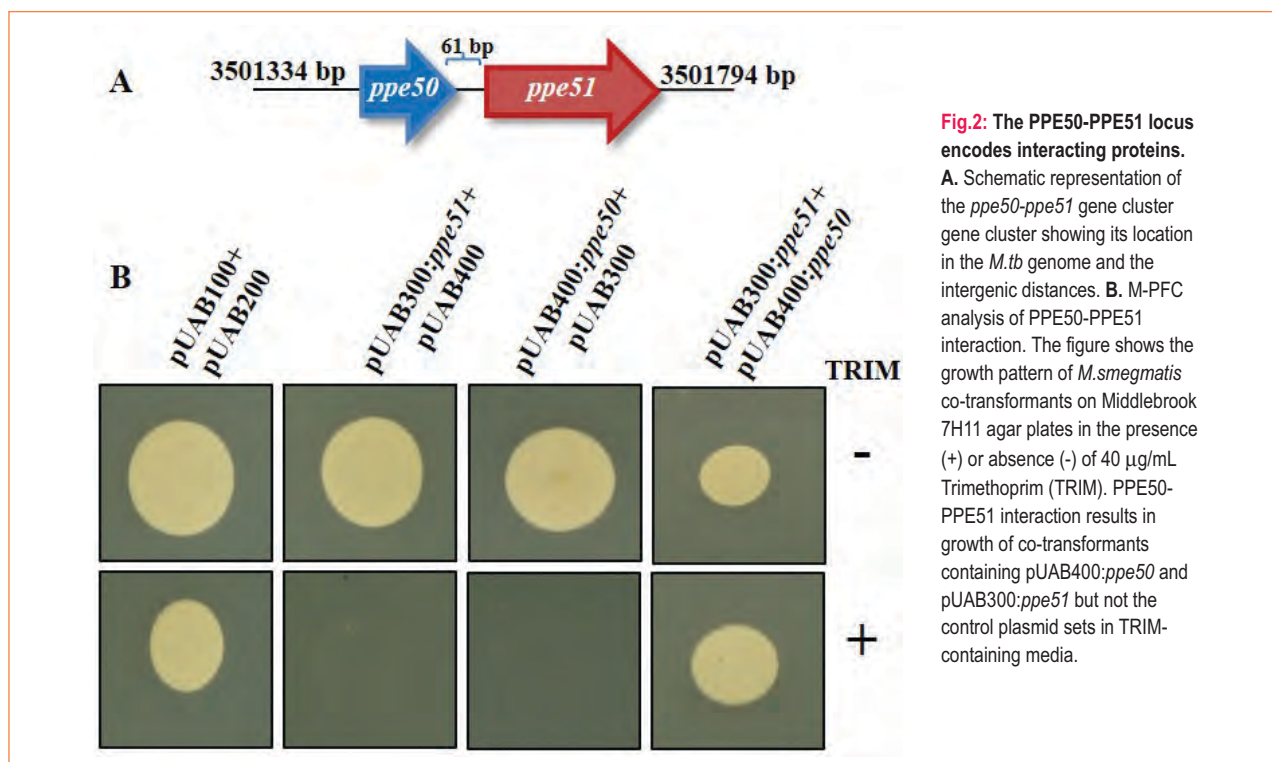


Fig.1: Binding of Ca²⁺ to M.tb DesA1.A.

Ca²⁺ overlay assay for DesA1 probed with radioactive ⁴⁵CaCl₂. Bovine Serum albumin (BSA) and Secretagoin were used as negative and positive controls respectively. The bar graph represents the pixel intensity of Ca²⁺ binding to the above proteins normalized to the loading controls. The data shown are representative of three independent experiments. **B.** Isothermal titration calorimetry of Ca²⁺ binding to DesA1. The concentrations of protein and Ca²⁺ used in ITC experiments were 50 µM and 5mM respectively. The protein in the sample cell was titrated with 45 injections of 6 µl each from a stock of 5µM CaCl₂. The table represents the binding kinetics of Ca²⁺ binding to DesA1. k_a macroscopic association constant, k_d dissociation constant, ΔH- change in enthalpy, ΔS – change in entropy.



in the pathogenesis of *M.tb*. We have extended this study and are now examining structure-function relationships in the desaturase and $\beta\gamma$ -crystallin domains of this protein.

Deciphering the role of the unusual *PPE50* (*Rv3135*) – *PPE51* (*Rv3136*) gene cluster in *M.tb* physiology

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 its role in *M.tb* physiology we have begun to characterise the *PPE50* (*Rv3135*)-*PPE51*

(*Rv3136*) gene cluster, one of four *PPE-PPE* clusters in the *M.tb* genome. Using RT-PCR we demonstrate that this cluster encodes a co-transcriptional unit and then hypothesised that this locus encodes interacting proteins, a common feature of operons. Using Mycobacterial Protein Fragment Complementation (M-PFC) we demonstrate that PPE50 and PPE51 interact *in vivo* (Figure 2). This observation was validated by *in vitro* pull-downs using purified preparations of these proteins. To examine the role of these proteins in host-pathogen interaction we expressed *PPE50* and *PPE51* as c-myc fusions in the surrogate saprophytic host *Mycobacterium smegmatis* and observed that they localise to the cell surface. 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. We are now performing cytokine profiling experiments to study the possible immunomodulatory functions of these proteins during infection.

Shrish Tiwari

Sequence Analysis of Biomolecules



From left to right : Vinay Rajput, Deepti Rao, Shrish Tiwari, Shubhankar Dutta, Parna Saha

RESEARCH INTERESTS:

- The analysis and evolution of the functional regions of the genome

We have done some previous work on *de novo* prediction of protein-coding genes in eukaryotic and prokaryotic genomes. Fine-tuning the parameters of the model according to the GC content of the region being analysed seems to improve our prediction accuracy and we are checking this out for genomes with different GC richness.

Selected recent publications

1. Bhardwaj A, Mukherji M, Sharma S, Paul J, Gokhale CS, Srivastava AK and Tiwari S (2009) "MtSNPscore: a combined evidence approach for assessing cumulative impact of mitochondrial variations in disease", *BMC Bioinformatics* 10 (Suppl 8):S7.
2. Paila Y D, Tiwari S, Sengupta D and Chattopadhyay A (2011) "Molecular modelling of the human serotonin_{1A} receptor: role of membrane cholesterol in ligand binding of the receptor", *Mol. Biosys* 7:224-234.
3. Chattopadhyay A, Paila YD, Shrivastava S, Tiwari S, Singh P and Fantini J (2012) "Sphingolipid-binding domain in the serotonin_{1A} receptor", *Adv. Exp. Med. Biol* 749: 279-93.
4. Rimpi Khurana, Vinod Kumar V, Abdul R, Tiwari S, Rekha A N, Ganesh M, M M Idris, Alan R C and Lekha D K (2014) "OncomiRdbB: a comprehensive database of microRNAs and their targets in breast cancer", *BMC Bioinformatics* 15:15.

We are getting into 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 in a web-based platform, MtSNP score that helps to prioritise SNPs which may be involved in mitochondrial diseases. MtSNP score 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 analysed two NGS datasets generated for rice. In one case an elite indica restorer line was introgressed with chromosomal segments of a wild rice using molecular markers and a major yield QTL from the wild rice was found to be associated with higher yield in the selected introgression line. The restorer line and one of the introgressed lines were sequenced and analysed to identify the introgressed regions. In the other project, resistance genes to blight infection from a wild rice were introgressed in sambhamahsuri (SM), a high quality rice from Andhra Pradesh. The resistant sambhamahsuri were selected and propagated to get the improved sambhamahsuri (ISM). SM and ISM (and a sister line of ISM (SISM)) were sequenced and analysed to identify regions of introgression. A protocol was developed based on SNPs to identify introgressed regions on all chromosomes. We are also in the process of analysing EMS mutant lines of rice with agronomically useful traits with the hope of identifying the genes responsible for the phenotype.

We have 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.

Tushar Vaidya

Molecular Analysis of Host-pathogen Interactions



From left to right : V Devi Prasad, Tushar Vaidya, Kaneez-e-Rabab, B Venkatesh, G Krishna Priya, Pradyumna Paranjape, Rathi Ranjan Kar, Satyajeeet Salunke, Loka Ram Prasad

RESEARCH INTERESTS:

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

Our laboratory is interested in understanding host-pathogen interactions that influence the outcome of infections.

Selected recent publications

1. Rudra Chhajer, Anirban B, Nicky D, Shadab M, Nirupam D, Partha P, Tushar V and Nahid A (2016) *Leishmania donovani* Aurora kinase: a promising therapeutic target against visceral leishmaniasis. *Biochem et Biophys Acta* 1860: 1973-1988.
2. Srijani B, Sheetal K, Lucas D'Souza, Tushar V, Vineeta B, Satyajit R and Anna G (2016) Constitutive CD40-signaling calibrates differentiation outcomes in responding B cells via multiple molecular pathways *J Immunol* 197:761-770.
3. Mala U, G. Krishna Priya, Ramesh P, Madhavi MB, Satyajit R, Vineeta B, Anna George and Tushar V (2014) CD40 Signaling Drives B Lymphocytes into an Intermediate Memory-Like State, Poised Between Naïve and Plasma Cells. *J Cellular Physiol* 229 (10):1387–1396.
4. Santosh K P, Sunil Kumar, Nitin C T, Tushar V, Anna G, Satyajit R, Vineeta B and Balachandran R(2012)Chitohexaose activates macrophages by alternate pathway through TLR4 and blocks Endotoxemia. *PLOS Pathogens* 8 (5):e1002717.
5. Vidhi P, Aneesh G, Sankaranarayanan R, Anton J E and Tushar V (2011) Evolutionary and functional insights into *Leishmania* META1: evidence for lateral gene transfer and a role for META1 in secretion. *BMC Evol Biol* 11: 334.

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 to identify and analyze molecules and mechanisms that confer any advantage to the parasite during the various stages of its life cycle. To facilitate our studies of these events, we have established a cell-free *in vitro* system for the growth and inter-conversion of promastigotes to amastigote forms of virulent *L. donovani*.

We have identified several genes that are differentially expressed in different stages of *Leishmania*. One such gene, META1, is a candidate virulence molecule. We have consistently observed that 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 and selection pressure on gene sequence, we have established that *Leishmania* META1 has been transferred by an ancient lateral gene transfer event between bacteria and a trypanosomatid ancestor. Superposition of META1 sequence on the solved structure of MxiM, a homolog in *Shigella* highlighted a putative hydrophobic cavity in META1. Mutagenesis of select hydrophobic residues in this cavity affects the secretion of the secreted acid phosphatase (SAP), indicating the involvement of META1 in secretory processes in *Leishmania*.

In ongoing experiments, we have observed that over expression of META1 causes a morphological reduction in size of *Leishmania*. In late stationary phase cells, greater than 90 % of META over expressing *Leishmania* are one-fifth the size of the wildtype cells. This is in contrast to just about 5% of wild type *Leishmania* undergoing a similar change. This phenotype is completely reversible; cells regain their size within 72 h, 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 over expression reduces the uptake and/or utilisation of nutrients. However, under all conditions tested, overexpression of META1 is necessary. In both wildtype and META1 over expressing *Leishmania* there is a 10-20 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 earlier shown by us to associate with increased secretion, diminishes the cells ability to undergo this transition. This, together with our observation of reduced nutrient uptake, underscores role for META1 in *Leishmania* secretory systems. Quite surprisingly, while over expression 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, 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 (DRG1 and DRG2) 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 have generated transgenic *Leishmania* ectopically expressing a) DRG1 b) DRG2 and c) DRG, just the common DRG sequence, lacking the respective specific elements. Preliminary *in vitro* infectivity studies with J774 macrophages and these transgenic *Leishmania* suggest that DRG1 over expression seems to enhance the internalization of *Leishmania* while over expression of the common DRG region alone seems to have a negative effect on the ability of *Leishmania* to infect macrophages. These findings suggest a role for DRG1 in the infective process and at the same time raises a possibility that the two paralog proteins cooperate to function.

Currently, we are studying 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, a RNA-Protein interaction prediction + CISBP, a Catalog of Inferred Sequence Binding Preferences). Using these combinatorial approaches, we have identified and are testing broad putative regulatory regions in DRG1 / DRG2 3' UTR. Once the cis-regions are identified, this work will be extended to identifying their differential binding protein partners through immunoprecipitation experiments and building an interaction map of the involved proteins.

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 signaling 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 signaling dominates over LPS signaling, 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 signaling 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. We are extending these studies by characterizing the cells post CD40

signaling along various parameters including cell surface markers, isotypes of Ig transcripts and longevity and survival. Additionally, we are studying the mechanisms by which CD40 signaling targets BLIMP1 down regulation.

In addition to the experimental approaches described above, we are currently in the process of assembling a Draft genome of the parasitic nematode *Setaria digitata*, a pathogen of significance in farm animals, and a comparative analysis with parasitic nematodes that infect humans. Genomic DNA isolated from individual worms was submitted for next generation sequencing. The Illumina Mi-Seq platform was used to generate paired end and mate pair reads from libraries ranging from 375bp, 500bp, 1.5kb and 5kb insert size. The assembly was improved by gap closing and extension of scaffolds using the bigger insert size libraries. The final assembly that we submitted to NCBI has the following statistics: Nucleotide size of ~118 MB, distributed over 17549 scaffolds, with N50 of 30415 and 14512 predicted genes.

This Venn diagram shows the distribution of shared gene families among *Setaria digitata*, three worms pathogenic to humans (*Brugia malayi*, *Loa loa* and *Onchocerca volvulus*), a non-pathogenic worm, *Caenorhabditis elegans* and an outlier organism, *Drosophila melanogaster*. The bar graph below Venn diagram indicates total clusters predicted for individual species (include unique clusters and clusters shared with two or more species). The bottom most color gradient pictorial representation demonstrates the total clusters unique to one organism, shared between any two, and so on.

Currently efforts are in progress towards annotation of the *Setaria* genome for predicted genes. KEGG pathway analysis using GhostKOALA has given us information on KEGG modules, enzymes and BRITE reconstruction pathways of *Setaria digitata*. We have also done the GhostKOALA run for *B.malayii*, *C.elegans*, *Bostaurus* (cattle), *Ovisaries* (sheep), *H.sapiens* (human). Comparing metabolic modules from the KEGG analysis of *S.digitata* with other nematodes, its own obligate host (bovine), aberrant host (Sheep) and Humans which it doesn't infect, are in progress for leads into key differences from other filarial worms. Additionally, Ortho Venn analysis has been done to identify ortholog clusters shared between *Setaria digitata* versus other animal pathogens -*Loa loa*, *B.malayii*, *O.volvulus*, non pathogenic *C.elegans* and *D.melanogaster*. *D.melanogaster* was selected as a well curated and annotated out group from Nematode genus. The

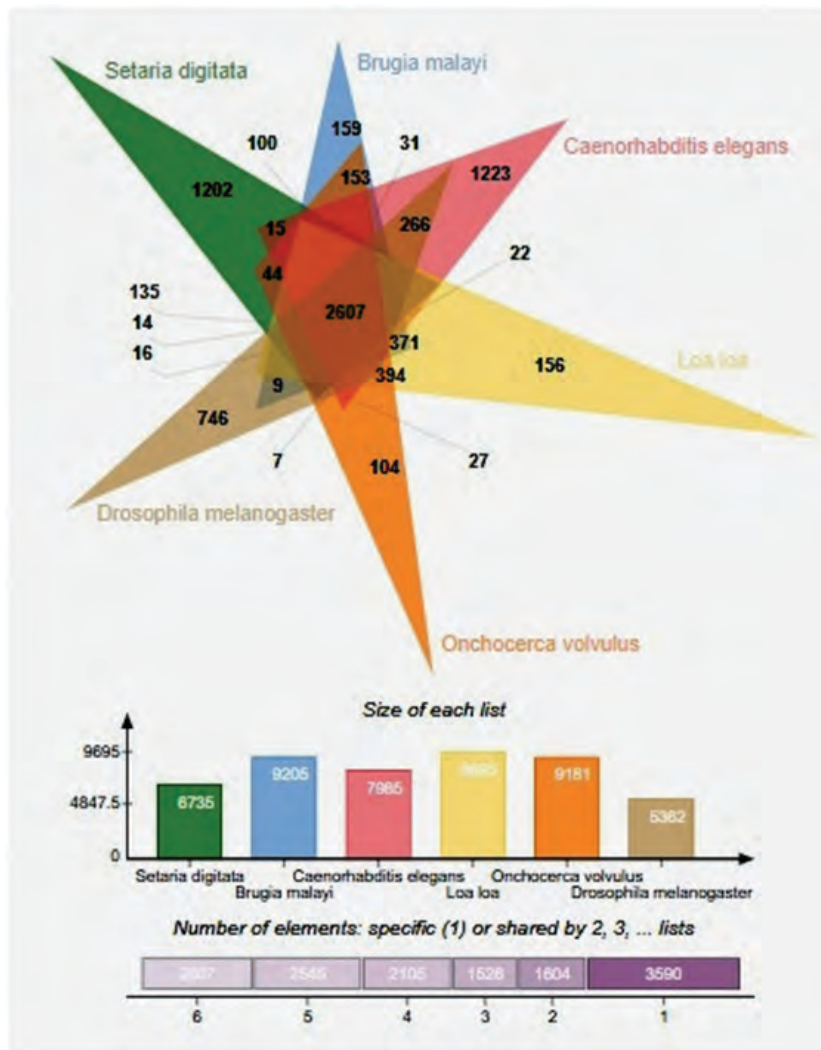


Fig.1: This Venn diagram shows the distribution of shared gene families among *Setaria digitata*, three worms pathogenic to humans (*Brugia malayi*, *Loa loa* and *Onchocerca volvulus*), a non-pathogenic worm, *Caenorhabditis elegans* and an outlier organism, *Drosophila melanogaster*. The bar graph below Venn diagram indicates total clusters predicted for individual species (include unique clusters and clusters shared with two or more species). The bottom most color gradient pictorial representation demonstrates the total clusters unique to one organism, shared between any two, and so on.

paralogs and orthologs clusters indicated many protein clusters of *S. digitata* that were majorly shared with other parasitic nematodes than free living *C. elegans*. Comparative analysis of the draft *Setaria* genome assembly with that parasitic nematodes that do infect humans such *B. malayi*, *W. bancrofti* will help understand their pathogenesis.

An interesting insight has been with regard to *Wolbachia* species, which are obligate bacterial endosymbionts essential for survival of the human

filial parasitic nematodes. The *Wolbachia* genome was downloaded from NCBI nucleotide database and local alignment of the genome was performed against the best SSPACE scaffold generated so far from the *Setaria* sequence, but we observed no significant hits against the *Setaria* genome. This suggests that *Setaria digitata* may not harbour *Wolbachia* as endosymbionts and may have evolved strategies distinct from the human parasitic nematodes to survive without *Wolbachia*.

Karthikeyan Vasudevan

Ecology and Conservation of Endangered Species



From left to right (front row) : Snehalatha Vadigi, Karthikeyan Vasudevan, Gayathri Sreedaran, Siddharth Bhatia
(back row): K Rajyalakshmi, Afsar Soghra, Ravi Kumar Singh, Milind C Mutnale, Ashish Jha

RESEARCH INTERESTS:

- The group works on ecology, evolutionary biogeography and systematics with a focus on south Asian amphibians and reptiles. Studies focussed on specific endangered reptile and amphibian species, on their ecology and conservation are being carried out.

Selected recent publications

1. Jegath Janani S, Vasudevan K, Perindini E, Dutta S K, Aggarwal R K (2017). A new species of the genus *Nasikabatrachus*. *Alytes* 34 (1-4): 1-19.
2. Mohanty N P, Harikirshnan S and Vasudevan K (2016). Watch out where you sleep: Nocturnal sleeping behaviour of Bay Island lizards. *PeerJ* 4:e1856;DOI10.7717/peerj.1856.

- Development of molecular assays for quantifying prevalence of chytridiomycosis in amphibians
- Developed robust methods to monitor biological invasion in Andaman Islands through key-informants
- Phylogenetic studies on bulbuls; egg-eating snake; gharial
- Quantifying geographic variation in saw-scaled venom constituents

The National Chambal Sanctuary in India holds the largest, most-stable and most-studied gharial subpopulation in the wild. This sanctuary stretches across the states of Rajasthan, Madhya Pradesh and Uttar Pradesh. The Gharial (*Gavialis gangeticus*) is endemic to the Indian subcontinent and Critically Endangered, and conservation work has been undertaken in the protected areas of Chambal. It is one among the longest lived reptilian species (up to 100 years) and spends half of its life as a reproductively active animal. The animal is also one of the largest crocodiles. The males grow 5-6 m, while the females grow to about 3.5-4.5 m. Since it has a prolonged pre-reproductive period, there would be no immediate apparent consequences of removal of breeding individuals. However the future ramifications would be a dramatic decline in their population size, hence the study of breeding biology of prime importance in gharials. We are focusing mainly on two key questions, which are 1) what is the kinship status in the breeding groups of gharials (big, medium, and small)? 2) is there multiple paternity in the communal nesting groups? To address these questions, we have collected about 3000 egg shell samples from a 200 km stretch of Chambal River (figure 1). We have covered nine communal nesting sites during the sample collection from 26 May to 15 June 2017. Back at LaCONES, we started isolating DNA from the egg membranes. The samples have yielded appreciable amount of DNA. We will use previously discovered microsatellite primers for gharials as well as Indian muggers, and screen them. We will be using the screened-in primers for genotyping of microsatellite markers using next generation sequencing. These sequences of microsatellite markers will unravel the breeding biology of gharials and will also serve as reference data for any further genetic study.

Gharials are one of the top predators present in the riparian ecosystem of the Chambal sanctuary. They feed on almost all organisms present in this system like molluscs, reptiles and fishes etc. It is one the largest



Fig.1: Gharial nest with freshly hatched out babies. This nest opened and assisted by our team to hatch out. (photo: Ravi Kumar Singh)

crocodiles present in India, which turns from a hatchling weighing a mere 100 grams to an adult weighing 1000 kilograms. Being a top predator, it feeds on large predatory fishes which in turn eat fishes of commercial value to the local people. In order to protect this species, it is very important to study the ecosystem functioning provided by this animal. We used stable isotope analysis techniques (using Stable Isotope Mass Spectrometry at CSIR-NGRI) to characterize the composition of stable carbon and nitrogen isotopes (expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). These measures define the items that are consumed as part of their diet. Specifically, tissue $\delta^{15}\text{N}$ compositions are a relative indicator of trophic position, and tissue $\delta^{13}\text{C}$ compositions reflect the habitats and food webs that animals use to acquire prey

We used tail scute clippings of the animals collected by GCA (Gharial Conservation Alliance) team. The samples were analysed in collaboration with CSIR-NGRI. We divided our samples into four categories based on body length, namely: juvenile, sub adult, adult female and adult male. We made a biplot of tissue $\delta^{13}\text{C}$ compositions (‰) and $\delta^{15}\text{N}$ compositions (‰). We found low $\delta^{13}\text{C}$ values and less variation in $\delta^{13}\text{C}$ as well as $\delta^{15}\text{N}$ values only among adult males, which suggests that the dietary niche of the adult gharial males is more specialized as compared to other categories. Less enrichment in $\delta^{13}\text{C}$ values is indicative of their food source being confined to those aquatic life forms occupying second or third trophic levels in food chain. On the other hand juveniles show maximum variation in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, this indicates that their diet forms a complex food web. Juveniles, sub adults and adult females also show huge variation in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, which points towards their more generalised dietary habit as compared to adult male. To sketch a comprehensive picture of gharial's dietary behaviour, we will need all the possible dietary samples from Chambal River, which will be accomplished in next couple of years.

Emerging Infectious Disease (EDI) is a threat to wildlife. One of such disease is Chytridiomycosis, considered to be a biodiversity disease capable of causing mass mortality events in global amphibian species. It is caused by two aquatic fungal pathogens *Batrachochytrium dendrobatidis* (Bd) and *Batrachochytrium alamanivorans* (Bs). Amphibians have highly permeable skin and are hence highly sensitive to changes in the environment making them ideal bio-indicators for the health of their surrounding ecosystem. Bd fungus colonies on the keratinous layer of frog skin and penetrate inside the skin epidermis to cause hyperkeratosis in frogs. The skin becomes thick-walled and interferes with gaseous exchange and osmoregulation. Very few

treatment options are available for Bd and Bs fungus. It is, therefore, necessary for effective surveillance to be able to detect the disease at its early onset in order to successfully conserve endemic frog species. We have limited data about the Bd fungus in India and only the Western Ghats has been surveyed for this fungus. The status of infection in other regions like The Himalayan region, North-East region, Eastern Ghats and Andaman Nicobar is unknown.

We used non-invasive swabbing technique wherein a cotton swab was firmly run 70 times over a frog's body. A new fresh pair of gloves was used in between animal to avoid cross contamination. Sampling was carried out in the Western Ghats, Eastern Ghats, North East India, Himalayan region and the Andaman and Nicobar Islands. DNA was extracted from the cotton swabs. A nested PCR was performed and PCR products were sequenced. All sequences were used to construct Bayesian tree and Median-joining haplotype network. A total of 1870 samples were collected from 142 locations comprising 30 genera, and 111 species. Nested PCR was used to screen all the samples. A total of 158 samples found to be positive for Bd fungus with prevalence 8.4%. Low prevalence was found in North East India with 0% prevalence while the Western Himalaya was leading with 14% prevalence. In the sequencing nested PCR product, two samples showed insertion of two adenine residues at the Taqman binding site while remaining two samples had transition and transversion mutation at Taqman binding site which can cause false negatives in qPCR analyses. We found a total of 57 haplotypes of which 46 were unique to India and 11 were shared between other countries. Two haplotypes namely IN02 and IN10 were prevalent in India. Both these haplotypes contributed 65% of total positive samples. Haplotype IN02 was most prevalent, represented in all the sampled areas (Figure 2). Haplotype IN10 was present in all sampled area except Western Himalayas (Figure 2). We constructed median joining network using all DNA sequences obtained in this study. The Indian haplotypes IN05 and IN55 containing mutations at the Taqman binding site were clustered together with the China haplotype CN30 and the Japan haplotypes JP09 and JP10.

The Yellow-throated Bulbul (YTB) is an endemic and threatened species found in small pockets of suitable habitats in thorny scrub forests across Eastern Ghats. Being endemic to the region with sparse and disjunct distribution, genetics of YTB populations secure signatures of ancient landscape level changes and geographic evolution. We are sampling YTB populations across its geographic range using mist netting (Figure 3) to understand the genetic differences between different populations. Also, we

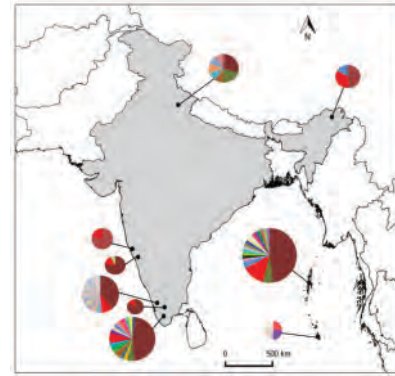


Fig.2: Bd haplotypes recovered from the different locations. Brown colored haplotype IN02 is wide spread throughout India. Red colored haplotype IN10 is present at all the location sampled except at Western Himalaya. Bd diversity was more at the Western Ghats.

are studying evolution of 24 species of Bulbuls from Indian Subcontinent. We are seeking answer to why is YTB endemic and rare while congeneric species are widespread and dominant. Our data shows that less than 100 populations of YTB are found across peninsular India with less than 1000 sq Km suitable habitat available. Preliminary phylogeny of Indian Bulbuls reveal that YTB does not have a sister species and has a unique evolutionary history. The ongoing genetic study of individual YTB populations will reveal if the populations are interconnected (signifying the ability of YTB to fly over to new habitats) or inbred (signifying reproductively isolated populations). Also the haplotype network will reveal if discrete YTB populations are a result of shrinkage of suitable habitat or in contrast, whether an ancient YTB population from one habitat dispersed and occupied suitable habitats around. It is expected that this work will push the cause of protection of scrub habitats across the Eastern Ghats and also the concept of preventive conservation for similar species.

Snake venoms contain complex mixtures of pharmacologically active molecules, including organic and mineral components, small peptides and



Fig.3: Mistnetting at Melukote and a bunch of keenly observant field staff and volunteers

proteins. These components can be grouped into a few major protein families, including enzymes (serine proteinases, Zn²⁺-dependent metalloproteases, and group II phospholipase A₂ iso-enzymes) and proteins with no enzymatic activity (C-type lectins and disintegrins). The biological effects of venoms are complex because different components have distinct actions and may, in addition, act in concert with other venom molecules. The synergistic action of venom proteins may either enhance their activities or contribute to the spreading of toxins. Snake bite poses a serious threat mainly in tropical countries including India with snake bite mortality estimated at 50000. The only effective treatment for systemic envenomation is the intravenous administration of an antivenom. Although antivenoms have gone a long way to reduce mortality, many of them do not achieve optimal protective effects. This is, in part due to the fact that conventional antivenoms are prepared from animal sera that are hyperimmunized with the whole venom. The resulting polyclonal antisera include numerous antibodies with specificities not confined to the toxic target molecules. Hence, knowledge of the toxin composition of venoms could be devised to generate GeneGun DNA immunization protocols to elicit toxin-specific antibodies with greater specificity and effectiveness than conventional systems.

Two to 3 mg of the crude venom was dissolved in 100 mL of 0.05% TFA and 5% ACN, and insoluble material was removed by centrifugation in an Eppendorf centrifuge at 13 000g for 10min at room temperature.

Proteins in the soluble material were separated using an Agilent system and Zorbax C18 column (250 X 4 mm, 5 mm particle size) eluted at 1mL/ min with a linear gradient of 0.1% TFA in water (solution A) and ACN (solution B). Protein detection was at 215nm and peaks were collected manually and dried in a SpeedVac (ScanVac).

The protein concentration was calculated for fraction using area under the peak as a measure. The RP isolated proteins fractions were then reconstituted with Tris-Cl and analyzed by SDS-PAGE. Protein bands for each fraction (as seen in gel below) were excised from a CBB-stained SDS polyacrylamide gel and subjected to reduction by DTT and alkylation with iodoacetamide, and digestion with sequencing grade bovine pancreas trypsin (Roche, Barcelona, Spain). The tryptic peptide mixtures were dried in a SpeedVac (ScanVac) and dissolved in 20 µL of 2% Formic Acid. For peptide sequencing, each protein fraction mixture was loaded in a nanospray capillary and subjected to ESI mass spectrometric analysis using a Q-Exactive mass spectrometer (ThermoFischer). Doubly- or triply-charged ions selected after Enhanced Resolution MS analysis were fragmented using the Higher-energy collisional dissociation (HCD). The HCD-MS/MS spectra was interpreted using Proteome Discoverer software. This approach allowed us to assign few isolated venom fractions to protein families present in the Swiss-Prot/TrEMBL non-redundant database as mentioned in Table 1.

HPLC FRACTION	m/z ion	MS/MS derived sequence	Protein family
4	1461(+3) 1553(+3) 1378(+3) 1023(+2)	EGEHcISGPccR NcKFLNAGTlcKK FLNAGTlcKKAR FLNAGTlcK	Metalloproteinases (Echis c. sochureki)
21	1523(+3) 852(+2)	AnEEDKGMVDEGTK GmVDEGTK	P-III Snake venom metalloproteinases
24	1586(+3) 1104(+2) 1232(+2)	KVLNEDEETREPK VLNEDEETR KVLNEDEETR	Serine Proteases

Table 1: CID-MS/MS raw data analyzed on proteome discover 1.4 using UNIPROT snake venom database. Sequest HT was used for obtaining hits to database. Carbamidomethylation was used as fixed modification and oxidation of methionine was used as variable modification for analysis. Carbamidomethylation was used as fixed modification and oxidation of methionine was used as variable modification for analysis.

Vegesna Radha

Signaling to the Cytoskeleton and Regulation of Cellular Functions



From left to right (front row) : Dhruv Kumar, Kunal Dayma, Vegesna Radha, Bh. Muralikrishna, Divya Sriram, Sanjeev Chavan Nayak, Ch. Ramulu.

RESEARCH INTERESTS:

- Regulation of cytoskeleton and cell differentiation
- Mechanism of action of cell death regulators

During the year we examined how C3G is regulated, and identified novel effectors that explain its essential role in maintaining cellular homeostasis. Dynamic nucleo-cytoplasmic exchange of C3G and its localization to nuclear speckles & the centrosome, suggest its role in regulating nuclear and centrosome functions. These findings advance our understanding of embryonic development as well as control of proliferation vs differentiation. Experiments using disease associated mutants of Ipaf have identified novel mechanisms of cell death signalling. These studies impact our understanding of normal cell functions at a molecular level and how deregulation can cause pathology.

Selected recent publications

1. Shakyawar DK, Dayma K, Ramadhas A, Varalakshmi C, Radha V(2017) C3G shows regulated nucleo-cytoplasmic exchange and represses histone modifications associated with euchromatin. *Mol Biol Cell* 28: 984-995.
2. Akhouri KR, Anand S, Gayathri G, Pendyala P, Yatender K, Radha V and Ghanshyam S (2017) A disease-associated mutant of NLRC4 shows enhanced interaction with SUG1 leading to constitutive FADD dependent caspase-8 activation and cell death. *J Biol Chem* 292(4):1218-1230.
3. Sasi K K, Ramadhas A, Nayak SC, Kaniyappan S, Dayma K, Radha V (2015) C3G (RapGEF1), a regulator of actin dynamics promotes survival and myogenic differentiation of mouse mesenchymal cells. *Biochim Biophys Acta- Mol Cell Res* 1853: 2629-2639.
4. Dayma K, Ramadhas A, Sasikumar K and Radha V (2012). Reciprocal negative regulation between the guanine nucleotide exchange factor C3G and β -catenin. *Genes & Cancer* 3:564-577.
5. Mitra A and V Radha (2010). F-actin binding domain of c-Abl regulates localized phosphorylation of C3G: Role of C3G in c-Abl mediated cell death. *Oncogene* 29:4528-4542.

Physiological processes such as tissue morphogenesis, wound healing, immune cell targeting, cell junction & shape maintenance, neuronal differentiation, adhesion and vesicle trafficking are dependent on cytoskeletal dynamics. A large number of human disorders are associated with defective cytoskeletal regulation. Our research focuses on characterizing regulatory molecules of intracellular signalling pathways leading to cytoskeletal remodelling, to understand how this fundamental process is controlled, and how deregulation results in pathological situations. We hope to identify molecular determinants regulating cell fate. These studies impact our understanding of normal development and maintenance of adult tissue integrity. Currently, our focus is on the guanine nucleotide exchange factor, C3G, which is ubiquitously expressed and functions in multiple signalling pathways. C3G regulates activation of small GTPases, Rap, R-Ras and TC10 and is known to have functions dependent on both catalytic and protein interaction domains. C3G is essential for mammalian embryonic development and many cellular functions in adult tissues. The molecular effectors and function of C3G in foetal and adult tissues are poorly defined. Studies in our laboratory showed for the first time an involvement of C3G in signaling to actin reorganization, and its role in differentiation. We have shown that C3G functions as a negative regulator of β -catenin signaling and may therefore control cell fate. How C3G regulates differentiation and other cellular functions is not fully understood.

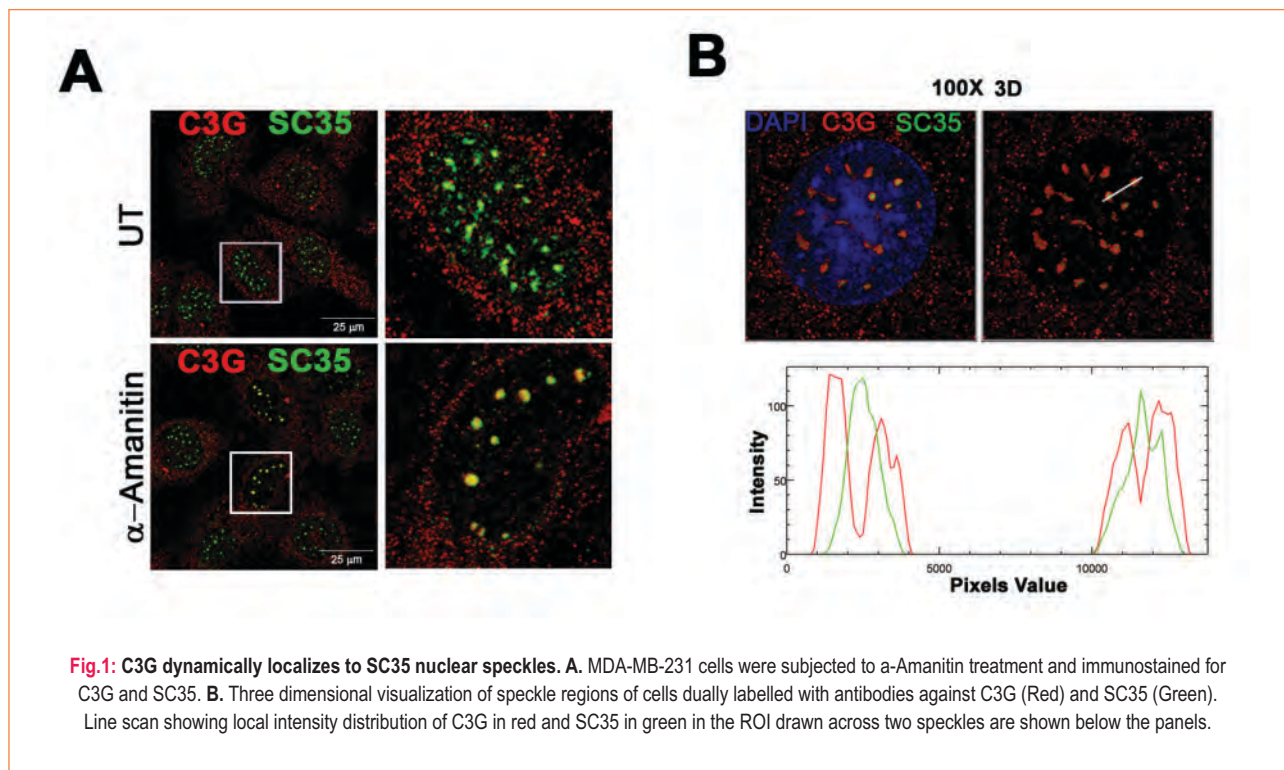
C3G was earlier known to localize exclusively to the cytoplasm. Our studies showed that dynamic nucleocytoplasmic exchange of C3G is regulated by functional NLSs and NES present in its primary sequence. GSK-3 β & okadaic acid sensitive phosphatases regulate dynamic exchange of C3G between cytoplasm & nucleus. In the nucleus, C3G represses histone modifications associated with euchromatin, and causes peripheralization of heterochromatin, indicating that C3G plays a role in regulating chromatin dynamics. This effect of C3G is mediated by histone deacetylases. Differentiation of myocytes and exposure of epithelial cells to sub-lethal doses of DNA damage induce nuclear translocation of C3G, and changes in chromatin. Using shRNA and CRISPR/Cas approaches, we

showed that C3G is required for maintaining histone modifications and expression of CDK inhibitors, p21 & p27. C2C12 clones lacking C3G, do not show upregulation of MHC, & do not fuse to form myotubes when grown in differentiation medium.

Reversible association of C3G with speckles was seen upon inhibition of transcription and splicing. C3G shows partial colocalization with SC35, and is recruited to a chromatin and RNase sensitive fraction of speckles. Its presence in speckles is dependent on intact cellular actin cytoskeleton, and is lost upon expression of the kinase, Clk1, and transient heat stress. Rap1, a substrate of C3G, is also present in nuclear speckles and inactivation of Rap signalling by expression of RapGAP, reduces speckle number. Enhanced association of C3G with speckles is seen upon GSK3 β inhibition, or differentiation of C2C12 cells to myotubes. CRISPR/Cas9 mediated knockdown of C3G resulted in decreased splicing activity and reduced staining for SC35 in speckles. C3G knockout clones of C2C12 as well as MDA-MB-231 showed reduced protein levels of several splicing factors compared to control cells. Our results identify C3G and Rap1 as novel components of nuclear speckles and a role for C3G in regulating cellular RNA splicing activity.

GSK3b interacts with, and phosphorylates C3G to retain it in the cytoplasm. *In vivo* and *in vitro* assays using phosphorylation site mutants showed multiple sites of phosphorylation by GSK3b. C3G over-expression represses, and knockdown of C3G enhances GSK3b activity. Examination of the expression of C3G in different cell types and functional regions of the brain showed high levels of expression in the dentate gyrus and in neuronal precursor cells. C3G expression was high in embryonic brain, even before neuronal differentiation is completed. Adult mouse olfactory bulb showed C3G expression in neurons but not in glial cells. In neurons, C3G shows predominant cytoplasmic localization.

In animal cells, the centrosome is the primary microtubule organizing centre, and is also the platform for primary cilium growth. These organelles serve as important signalling centres, and defects in their structure or function is associated with many human disorders. Recent findings show that endogenous C3G localizes to the mother centriole, is



present at the base of primary cilium (Figure 1), and interacts with cenexin. In prophase cells, C3G is seen at only one of the centrosomes, and in later phases of mitosis, is present in both the centrosomes, suggesting that it marks the mother centriole at the end of prophase. Knockdown of C3G showed that it plays a role in regulating centrosome duplication and primary cilium formation. We are currently addressing how C3G regulates centrosome functions.

In collaboration with Dr. GhanshyamSwarup, we have shown that one of the autoinflammatory syndrome-causing mutants of NLRC4, H443P, but not T337A and V341A, constitutively activates caspase-8 and

induces apoptotic cell death in human lung epithelial cells. Compared to wild type NLRC4, the H443P mutant shows stronger interaction with SUG1 and with ubiquitinated cellular proteins. Phosphorylation of NLRC4 at Ser533 plays a crucial role in caspase-8 activation and cell death. However, H443P mutant does not require Ser533 phosphorylation for caspase-8 activation and cell death. Our findings show that the auto-inflammation associated H443P 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.

Sunil Kumar Verma

Signal Transduction Studies in Human Health and Disease and Molecular Biology Applications in Wildlife Conservation



From left to right : Sanjay Kumar Suman, Sunil Kumar Verma, Sampurna Balde, Megha Kumari, R. Siva Dharshini, Sanjana Shirva, Nabanita Biswas

RESEARCH INTERESTS:

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

Selected recent publications

1. 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).
2. Nayak A, Salt G, Verma S K and Kishore U (2015). Proteomics Approach to Identify Biomarkers in Neurodegenerative Diseases. *Int Rev Neurobiol* 121:59-86.
3. Verma S K and Sarkar M K (2015) *Decalepisarayaalpathra* (J. Joseph & V. Chandras.) Venter, an endemic and endangered ethno medicinal plant from Western Ghats, *India. Nat Prod Res* 29:394-395.
4. Verma S K and Goswami G K (2014) DNA evidence: Current perspective and future challenges in India. *Forensic Sci Int* 241:183-189.
5. Verma S K, Ganesan T S, Kishore U and Parker, P. J. (2011). The tumour suppressor RASSF1A is a novel effector of small G protein Rap1A. *Protein Cell*, 2:237-249.

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

The “Universal Primer Technology” invented by us in the past became a milestone in the area of Wildlife Forensics, in particular the DNA based Identification of species to satisfy legal evidence requirements in a court of law. This invention became instrumental to solve more than 1300 cases pertaining to wildlife crime. However, due to absence of any training facility in the country on the application part of it, the beneficiaries of this invention such as Wildlife Officers, IPS, IFS, Police personnel and Judiciary lagged behind and could not update their skills in this arena. In this year, we have started a unique program under the skill India mission of Government of India which aims to fill these gaps by establishing a state of the art National Wildlife Forensics Training Laboratory in the LaCONES of CCMB (Figure 1).

With the increased practical implications of advanced DNA technologies in various sectors, in particular the judiciary, police, wildlife and forest and most recently the food safety departments, this programme will be another milestone in developing our national capability in this area for proper implementation of India's wildlife forensics and conservation efforts.

Other than this my laboratory is also playing a pivotal role in establishing the yet unexplored arena of wild flora forensics to help combat India with illegal plant trade as well as to identify and protect the national wealth of our medicinal plants.



Fig.1: Illustration of various stakeholders who will benefit from this skill development program.

- (i) Officers working in establishments under various departments, subordinate offices, attached offices, autonomous bodies and organizations, PSUs and missions of various Ministries of Government of India, such as:
 - Ministry of Environment, Forests and Climate Change (MoEF)
 - Ministry of Law and Justice (MoLJ)
 - Ministry of Commerce and Industry (MoCI)
 - Ministry of Food Processing Industries (MoFPI)
 - Ministry of Health & Family Welfare (MoHFW)
 - Ministry of Home Affairs (MoHA)
 - Ministry of Agriculture and Farmers Welfare (MoAFW)
 - Ministry of Finance - Department Of Revenue
- (ii) Academic, Research Institutions and Universities: Training of the trainers and teachers
- (iii) Licensees and interested parties, including private and public prosecutors willing to upgrade their skill on DNA based interventions
- (iv) Students interested in taking the above arena as their career plan



List of Publications and Patents
PhD Program
Training Programs
Research Facilities
Research Resources
Services
CCMB Innovation Hub

III.1 List of Publications and Patents (2016-2017)

1. Ahmad M, Nongmaithem SS, Krishnaveni GV, Fall CHD, Yajnik CS and Chandak GR (2016) Lack of replication of association of THSD7A with obesity. *Int J Obesity* 40(4):725-726.
2. Ahn TY, Kang JH, Kang DJ, Venkatesan J, Chang HK, Bhatnagar I, Chang KY, Hwang JH, Salameh Z, Kim SK, Kim HT and Kim DG (2016) Interaction of stem cells with nano hydroxyapatite-fucoidan bionanocomposites for bone tissue regeneration. *Int J Biol Macromol* 93:1488-1491.
3. Ahsan SM and Mohan RC (2017) The role of surface charge in the desolvation process of gelatin: implications in nanoparticle synthesis and modulation of drug release. *Int J Nanomed* 12:795-808.
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Patents (2016-17)

Patents filed Abroad

1. Production of metal nanoparticles in aqueous solution. Sankalp Vinod Agarwal, Shyam Sunder Reddy, Marshal. Filed in Canada on 2 May 2016. Patent Appln No.292943,1 NF No.0230NF2013/CA
2. Production of metal nanoparticles in aqueous solution. Sankalp Vinod Agarwal, Shyam Sunder Reddy, Marshal. Filed in US on 2 May 2016. Patent Appln No.15/033741, NF No. 0230NF2013/US
3. Production of metal nanoparticles in aqueous solution. Sankalp Vinod Agarwal, Shyam Sunder Reddy, Marshal. Filed in Australia on 3 May 2016. Patent Appln No. 2014343178, NF No.0230NF2013/AU
4. An International Patent (CCMB, NIIST) on Novel Porphyrin derivatives for Photodynamic Therapy (PDT): A process for the preparation thereof and their use as PDT agents and fluorescence probes for biological applications. Danoboyina Ramaiah, Suneesh C Karunakaran, Vadakkancheril S Jisha, Tavarekere K Chandrashekhara, Alagar Srinivasan, Madhavan Radhakrishna Pillai, Sivakumari Asha Nair, Saneesh Babu P Saras, Chintalagiri Mohan Rao, Kunchala Sridhar Rao. Filed in Denmark on 4th May 2016. Patent Appln No. 11711133.6, NF No. 0147NF2009/DE
5. Novel Porphyrin derivatives for Photodynamic Therapy (PDT): A process for the preparation thereof and their use as PDT agents and fluorescence probes for biological applications. Danoboyina Ramaiah, Suneesh C Karunakaran, Vadakkancheril S Jisha, Tavarekere K Chandrashekhara, Alagar Srinivasan, Madhavan Radhakrishna Pillai, Sivakumari Asha Nair, Saneesh Babu P Saras, Chintalagiri Mohan Rao, Kunchala Sridhar Rao. Filed in France on 4th May 2016. Patent Appln No. 11711133.6, NF No. 0147NF2009/FR
6. Novel Porphyrin derivatives for Photodynamic Therapy (PDT): A process for the preparation thereof and their use as PDT agents and fluorescence probes for biological applications. Danoboyina Ramaiah, Suneesh C Karunakaran, Vadakkancheril S Jisha, Tavarekere K Chandrashekhara, Alagar Srinivasan, Madhavan Radhakrishna Pillai, Sivakumari Asha Nair, Saneesh Babu P Saras, Chintalagiri Mohan Rao, Kunchala Sridhar Rao. Filed in Great Britain on 4th May 2016. Patent Appln No. 11711133.6, NF No. 0147NF2009/GB
7. Biomarkers useful for detection of grades of human breast cancer. Dinesh Kumar Lekha, Verma Vinod Kumar, Appukuttan Nair Rekha, Jem Prabhakar, Katoor Jayasree. Filed in Great Britain on 19th May 2016. Patent Appln No. 1608839.5, NF No. 0037NF2011/GB
8. Production of metal nanoparticles in aqueous solution. Sankalp Vinod Agarwal, Shyam Sunder Reddy, Marshal. Filed in Europe, on 26th May 2016. Patent Appln No. 14821300.2, NF No. 0230NF2013/EP
9. Production of metal nanoparticles in aqueous solution. Sankalp Vinod Agarwal, Shyam Sunder Reddy, Marshal. Filed in China 24th June 2016. Patent Appln No. 2.0148E+11, NF No. 0230NF2013/CN
10. Recombinant protein-based method for the delivery of silencer RNA to target the brain. Gopal Vijaya, Ghulam Hassan Dar. filed in US on 15th Sep 2016. Patent Appln No. 15/266642, NF No. 0147NF2016/US
11. An International patent (PCT), Novel Chlorine based compounds, a process for preparation thereof and use as photodynamic therapeutic agents and fluorescent probes. Marydasa Betsy, Bollapalli Madhuri, Danoboyina Ramaiah, Kunchala Sridhar Rao, Chintalagiri Mohan Rao, Tavarekere Kalliah Chandrashekhara, Suneesh Chandramathi Karunakaran. Filed at PCT on 21 Sep 2016. Patent Appln No. PCT/IN2016/050318, NF No. 0093NF2015/WO
12. An international Patent (PCT), Protein nanostructures for the delivery of therapeutic agents to the anterior segment of the eye. Saad Mohammad Ahsan, Chintalagiri Mohan Rao. Filed on 16 Nov 2016. Patent Appln No. PCT/IN2016/050400, NF No. 0153NF2015/WO.

Patents granted

1. 2526103 [Denmark(DE)]: Novel porphyrin derivatives for photodynamic therapy (pdt): a process for the preparation thereof and their use as pdt agents and fluorescence probes for biological applications. Danoboyina Ramaiah, Suneesh C Karunakaran, Vadakkancheril S Jisha, Tavarekere K Chandrashekhara, Alagar

- Srinivasan, Madhavan Radhakrishna Pillai, Sivakumari Asha Nair, Saneesh Babu P Saras, Chintalagiri Mohan Rao, Kunchala Sridhar Rao. Granted on 04-May-16.
2. 2526103 [France(FR)]: Novel porphyrin derivatives for Photodynamic Therapy (PDT): a process for the preparation thereof and their use as pdt agents and fluorescence probes for biological applications. Danoboyina Ramaiah, Suneesh C Karunakaran, Vadakkancheril S Jisha, Tavarekere K Chandrashekhar, Alagar Srinivasan, Madhavan Radhakrishna Pillai, Sivakumari Asha Nair, Saneesh Babu P Saras, Chintalagiri Mohan Rao, Kunchala Sridhar Rao. Granted on 04 May 2016.
 3. 2526103 [Great Britain]: Novel porphyrin derivatives for Photodynamic Therapy (PDT): A process for the preparation thereof and their use as pdt agents and fluorescence probes for biological applications. Danoboyina Ramaiah, Suneesh C Karunakaran, Vadakkancheril S Jisha, Tavarekere K Chandrashekhar, Alagar Srinivasan, Madhavan Radhakrishna Pillai, Sivakumari Asha Nair, Saneesh Babu P Saras, Chintalagiri Mohan Rao, Kunchala Sridhar Rao. Granted on 04 May 2016.
 4. 2526103 [PCT]: Novel porphyrin derivatives for photodynamic therapy (PDT): a process for the preparation thereof and their use as pdt agents and fluorescence probes for biological applications. Danoboyina Ramaiah, Suneesh C Karunakaran, Vadakkancheril S Jisha, Tavarekere K Chandrashekhar, Alagar Srinivasan, Madhavan Radhakrishna Pillai, Sivakumari Asha Nair, Saneesh Babu P Saras, Chintalagiri Mohan Rao, Kunchala Sridhar Rao. Granted on 04 May 2016.
 5. ZL201280034542.X [China]: Wdr13 as a novel biomarker useful for treating diabetes and cancer. Satish Kumar, Vijay Pratap Singh. Granted on 31 Aug 2016.
 6. 2536374 [Great Britain] Biomarkers useful for detection of grades of human breast cancer. Lekha Dinesh Kumar, Vinod K verma, Rekha A nair, JemPrabhakar, Jayasree Kattoor. Granted on 26 October 2016.

Academic Cell [PhD (JNU & AcSIR) Program]

The CCMB-PhD program targets students who intend to pursue research careers in interdisciplinary areas within or outside academia. Our aim 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 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 Academic Cell, which consists of two Academic Coordinators, an Academic In-charge, and an assistant. This cell handles all the

academic activities related to PhD students, including selection twice a year in January and August and recruitment of students, course work, lab allotment and the meetings of doctoral advisory committee (DAC). The Academic Cell keeps record of performance in course work, progress reports of the PhD work, and all AcSIR related documents. In case of JNU-CCMB PhD program, JNU-CCMB committee takes care of its administrative matters. The students can apply through CCMB-JNU, CCMB-ACSIR and CCMB-JGEEBILS streams. 16 students were selected for August 2016 and 5 students were selected for January 2017 PhD programs.

III.2 PhD PROGRAM

List of Students awarded PhD degrees during the period April 2016 to March 2017

1. **Jaya Krishnan**
Genome organization chromatin structure and gene regulation: Role of simple sequence repeats (04-04-2016)
Guide: **Dr. Rakesh K Mishra**
2. **Saad Mohammad Ahsan**
Micro/nano-particles in ophthalmic drug delivery (05-04-2016)
Guide: **Dr. Ch. Mohan Rao**
3. **Salil Saurav Pathak**
Study of epigenetic mechanisms in depression associated neuroadaptations in brain reward circuitry: Role of few Jumonji domain containing histone H3 demethylases (26-04-2016)
Guide: **Dr. Arvind Kumar**
4. **Dipesh Kumar Singh**
Functional analysis of chromosome organization in plants: The role of AtCTF7 and PATRONUS1 in meiotic sister chromatid cohesion (16-05-2016)
Guide: **Dr. Imran Siddiqi**
5. **Basil Mathew**
Insights into the structure - activity relationships of human defensins (31-05-2016)
Guide: **Dr. R Nagaraj**
6. **Ashaq Hussain**
Role of exoribonuclease R and RNA helicases in low-temperature adaptation of the Antarctic bacterium *Pseudomonas syringae* (09-06-2016)
Guide: **Dr. Malay K Ray & Dr. N M Rao**
7. **S Durgesh Kumar**
Signal transduction in a terminally differentiated mammalian cell: Functional characterization of candidate proteins in Hamster Spermatozoa (04-07-2016)
Guide: **Dr. Archana B Siva & Dr. S Shivaji**
8. **Megha Bansal**
Regulation of signal transduction and autophagy by optineurin (22-07-2016)
Guide: **Dr. Ghanshyam Swarup**
9. **Sai Chaitanya Chiliveri**
Structure-function relationship of dsRNA binding proteins involved in RNAi pathway of *C. elegans* and *A. thaliana* (09-08-2016)
Guide: **Dr. Mandar V Deshmukh**
10. **Debaditya De**
Functional analysis of nuclear matrix components in *Drosophila Melanogaster*: Role of boundary element associated factor (BEAF) in cell cycle (17-09-2016)
Guide: **Dr. Rakesh K Mishra**
11. **Bal Krishnan Mishra**
Diverse facets of non-lens $\beta\gamma$ -crystallins: biophysics and biology (24-10-2016)
Guide: **Dr. Yogendra Sharma**
12. **Shiladitya Mitra**
Molecular analysis of WDr13 gene function (25-11-2016)
Guide: **Dr. Satish Kumar**
13. **K Priyadarshan**
Structural and mechanistic basis of fatty-acyl activation for lipid metabolism in *Mycobacterium* (16-11-2016)
Guide: **Dr. R Sankaranarayanan**
14. **Ashutosh Shukla**
Relation of Histone variant H2A.Z with the RNA polymerase III transcription (27-12-2016)
Guide: **Dr. Purnima Bhargava**
15. **Rahul Navale**
Variant antigens and autophagy proteins of malaria parasites: roles in parasite development and virulence (13-01-2017)
Guide: **Dr. Puran Singh Sijwali**
16. **Satya Brata Routh**
Exploring the mechanism of chiral proof-reading during translation of the genetic code (25-01-2017)
Guide: **Dr. R Sankaranarayanan**
17. **Nikhil Sharma**
Study on Japanese encephalitis virus (JEV) neuropathogenesis (15-02-2017)
Guide: **Dr. Puran Singh Sijwali & Dr. Sunit K Singh**
18. **T Lavanya**
Functional studies on *Xanthomonas oryzae* pv. *Oryzae* Type 2 secretion system secreted proteins (09-03-2017)
Guide: **Dr. Ramesh V Sonti**
19. **Gopinath V**
Identifying virulence genes of *Mycobacterium tuberculosis*: A mutagenesis based approach using *Mycobacterium smegmatis* (31-03-2017)
Guide: **Dr. Raghunand Tirumalai**

III.3 Training Programs

Summer Training Program

The Summer Training Program of CCMB was started to provide students a real time working opportunity and encourage young students to pursue science as a career option comprising serious research training for 8 weeks during the summer months. The program has become a pan-India endeavour with applications from across the country. During 2012-2017, 418 summer interns were trained at CCMB. This year we again invited applications where more than 1000 students in their final year of Master and BTech courses applied from across the nation. A total of 84 applicants were selected wherein 73 students joined, including 01 from our neighbouring nation, Nepal for this 2-months' program during May-July 2017. We make serious efforts to run this program successfully wherein our intention is to provide students a real time working opportunity in a vibrant research laboratory.

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 CCMB under the supervision of a Scientist towards their partial fulfilment 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, candidates are exposed to recent research developments, scientific ethics, good laboratory practices and career opportunities in life sciences. At the end of the training, candidates will be

presenting their work in the form of posters to the scientific community at CCMB. Ever since the program is formalized under skill initiative in June 2017, we have received 350 applications from all over the country and 69 candidates were selected by the end of September 2017.

Skill Development Program at CCMB

Centre for Cellular and Molecular Biology (CSIR-CCMB), since its inception, has been committed to training of human resource in the advanced areas of biology to serve the needs of development in these areas. This has been propelled further by the initiation of the CSIR-Integrated Skill Initiative, under whose ambit this year CCMB initiated the Skill Development Program (SDP) in Biological Sciences. As an initial step, dedicated Skilling Labs were made-ready with equipment meant for running the offered courses.

The Skilling courses offered are in the areas of Cell Biology, Wildlife Forensics and Bioinformatics. (<http://www.ccmb.res.in/index.php?view=training&mid=0&id=73&nid=121>)

Another key addition to the SDP activities at CCMB was the training program conceived on "Research Methodologies for Medical Students (MedSRT)". This, in addition to other collaborative activities; was as a part of the MoU signed between CCMB and the Directorate of Medical Education (DME), Telangana State. The 1st MedSRT training was held from 8th May - 8th June 2017 and this also in a way marked the formal launch of the CCMB's Skill Development Program.



III.4 Research Facilities

CCMB has a number of facility groups consisting of scientists and technical staff with expertise in specific techniques in biology and biochemistry. They provide support to various in-house ongoing research programs and also extend help to other institutions all over the country. Some of the fully functional major facilities are described below:

Advanced Microscopy and Imaging Facility

Confocal Microscopes

Confocal microscope scans specimens and allows data collection in 3-Dimensions. This facility uses inverted microscopes with various objectives and receives illumination from various laser lines. The systems are provided with facilities for applications like Scanning and analysis of single and multi labelled samples combined with DIC, 3D Reconstruction, Kinetic Analysis, Ratio Analysis, Spectral Analysis, Fluorescence Recovery after Photo bleaching (FRAP) and fluorescence resonance energy transfer (FRET).

Multiphoton confocal microscopy system

It has the IR-laser (Chameleon Ultra II), which allows greater penetration with minimal photobleaching and Non-descanned detectors (NDD) to efficiently collect fluorescence from specimens that scatter light strongly such as brain sections.

Live Cell Laser Scanning Confocal Microscopy System for real time imaging

While maintaining samples at temperatures ranging from ambient to 40°C with CO₂ supply, this system can be used for FRET, FRAP, Multiple time series experiments.

High Resolution and Sensitive Laser scanning Confocal Microscope for Imaging Biological Samples

The system is used for the scanning and analysis of

single and multi labeled samples combined with DIC, 3D Reconstruction, Kinetic Analysis, Ratio Analysis, Spectral Analysis, Fluorescence Recovery after Photo bleaching (FRAP), Fluorescence Resonance Energy transfer (FRET), Anisotropy and Fluorescence Correlation Spectroscopy (FCS).

In addition, the facility also has the following Advanced Microscopes:

1. Universal research microscope model Axioplan 2 imaging
2. Apotome Fluorescence Imaging system with fully motorized Axioimager.Z1 Microscope and monochrome digital camera
3. Fluorescence Stereo Microscope with 3D system and color CCD camera
4. Total Internal Reflection Fluorescence Microscopy System
5. Live cell-imaging system
6. Advanced Fluorescence Stereo Microscope with Apotome
7. Atomic Force Microscope (AFM)
8. Raman Spectroscopy And Raman Rapid Imaging

Apart from the above the facility also has Transmission and Scanning Electron Microscopes (**TEM and SEM**)

This year, 206 samples were analysed using TEM and 290 samples analysed using SEM facility. Samples from other institutes were also analysed on payment basis.



Animal House Facility



The CCMB Animal House was established in 1987 with the main objective of supplying defined strains of mice, rats, hamsters and rabbits to the scientific community of the centre for research under strict regulatory oversight. 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 Animal House 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 45 strains of mice including transgenic & knockout mouse models, immuno-compromised (SCID and 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. 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 Institutional Animal Ethical Committee in this year is 137.

Brain & Behaviour Facility

Brain and Behavioral Facility at CCMB is fully equipped with adequate instrumentation to carry out behavioral phenotyping of transgenic animals at CCMB and to perform behavioral neuroscience experiments to investigate the etiopathology of neuropsychiatric disorders using diverse mouse and other rodent models. The facility is integral to the interdisciplinary efforts of CCMB and has been

catering to the requirements of researchers within the institute as well as across the country for the behavioral and neuropharmacological analysis in animals. This state of the art facility has been instrumental in advancing fundamental work in basic neuroscience and also for expanding the horizon of translational efforts towards the overall mental health and neurodegenerative disorders viz., depression, stroke, Alzheimer's and vascular dementia.

BSL 2 & 3 Laboratories

The Biosafety levels 2 and 3 laboratories provide a safe working environment for high risk and high-security microbial pathogens, where trained users handle various pathogenic microbes. The labs cater to the infrastructural requirements of research groups in CCMB working in the areas of virology, bacteriology and parasitology. The laboratory design and standard operating procedures protect the user, environment, and the community from any risk. Each level of containment has specific microbiological practices, safety equipment and facility safeguards for the corresponding level of risk associated with handling of a particular agent.



Cell Culture Facility

The centralized Cell Culture Facility of CCMB caters to the need of all groups in CCMB that uses cultured 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



From left to right: Srinivas, P Venugopal, T Dayakar, B V V Pardhasaradhi, S Easra, Zareena Begum, Ch Varalakshmi, Pulamma, Dr V Radha

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, CO2 incubators, inverted microscopes, freezers, cold storage, liquid nitrogen storage facility, Fluid cell imaging system, electroporator, nucleofector, automated cell counters, photo-dynamic therapy instrument, hypoxia chamber, etc. 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, organ explant and primary cultures, cell fusion to produce Monoclonal antibodies, DNA transfection to establish stable clones and cryopreservation of cells. 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. We ensure that the lines are validated to be free of contamination. This year we have introduced a short term training course on Animal Cell Culture for students/Faculty/Researchers from Universities/ Institutes/Industry interested in learning cell culture techniques.

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 AffymetrixGeneChip System for analyzingAffymetrix Chips and the IlluminaHiScan System for sensitive and accurate imaging of Illumina BeadArrays 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 for users within CCMB and also to users outside CCMB.



FACS Facility

The CCMB FACS facility is centralized facility equipped with two high-speed sorters and two high-speed analysers. The facility provides training to students/project staff as well as support in design and analysis of experiments to all CCMB investigators and to the local scientific community outside CCMB. The facility has a 3 Laser sterile sorting enabled MoFlo-XDP with class II Biosafety cabinet and non-sterile sorter MoFlo-Legacy. A high speed 3 Laser Galliosanalyzer with auto loader caters to the analysis of experiments.

FLY LAB



Drosophila melanogaster, the Fruit fly is one of the most studied organisms in genetics research. Almost a third of known human diseases have a match in fruit flies, and over half their proteins are similar to human. That's why studying patterns of inheritance of certain fruit fly traits can be so valuable to humans. They're also important for genetic studies because fruit fly mutations are often easily observed.

In CCMB fly lab we maintain and stock about 1500 different fly strains for ongoing research activities including the studies of body patterning, development, stress, longevity. We also maintain transgenic fly for human disease model which are used for drug screening. This includes Parkinson's disease and Alzheimer's disease. We have full-fledged imaging setup for the bright field and fluorescent samples. Students and teaching staff from different universities visit fly lab to get the hands-on experience to culture and maintain the flies. Fly lab of CCMB provides flies to different colleges in the city for teaching 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 supports a wide range of projects of the research groups at CCMB.

NMR Micro-imaging And Spectroscopy Facility

A. Widebore 600 MHz NMR Microimager and Spectrometer

In this system, a 600 MHz Avance II Microimager and Spectrometer is interfaced with a wide bore (89 mm) 14.1 T magnet system. The Microimager is equipped with actively shielded mini and micro probes with maximum gradient strength of 150 Gauss/cm, which provides in vivo images at micron resolution. The system is equipped with several volume coils (^1H , ^{13}C , ^{31}P) for in vivo imaging and spectroscopic study with mice and rats. This provides an opportunity to acquire 20-30 images in short time. Moreover, localized in vivo NMR spectroscopy could be carried out from a voxel of $2\text{X}2\text{X}2\text{ mm}^3$ in mice brain. Additionally, the spectrometer is equipped with high resolution triple resonance and broad band probes for detection of X-nuclei (^{13}C , ^{19}F , ^{31}P) for NMR analysis in solution. CCMB Scientists are using the current setup to identify subtle changes in brain atrophy, and understanding neurometabolites homeostasis, and energetics of excitatory and inhibitory neurotransmitters in different neurological and psychiatric disorders like Parkinson's, Alzheimer's, Amyotrophic sclerosis, Depression, Addictions, etc using different models. Additionally, the setup is used for development of MRI contrast agents.

B. 600 MHz NMR Spectrometer

The 600 MHz narrow bore NMR facility was setup in 2009 with an aim to study biomolecular structure and function at the physiological condition in the solution. The spectrometer is equipped with a cryogenically cooled probe. The enhanced sensitivity of the cryoprobe allows de novo 3D structure determination of relatively large proteins ($\text{MW} > 25\text{ kDa}$) and nucleic acids as well as their ligand bound complexes at the physiological condition. The spectrometer is routinely used to derive biologically relevant conformational flexibility of proteins and nucleic acids in situ. Over the years, the NMR spectrometer has become an integral part of CCMB's research activities and had immensely contributed to numerous projects including studies on solution structures like RDE-4, DRB4, Crc ($\sim 32\text{ kDa}$) and their interactions with RNA; understanding the mechanism of chiral proofreading during protein translation; studies and design of

thermostable Lipases, studies on antimicrobial peptides and interaction of intracellular loops of GPCRs with membranes, structure-function relationship of key proteins in *P. falciparum*.

Proteomics Facility



The Proteomics Facility of 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, OrbitrapVelos, and MDS SCIEX 4800 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.

Radio Isotope Facility

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

Transgenic and Gene Knock out Facility (TGKF)



The Transgenic and gene knockout mice core facility was established to create, procure and maintain, Transgenic and gene knockout mice models. Gene targeting in embryonic stem cells, micromanipulation, survival surgeries are performed to generate Transgenic and gene knockout mice models. The facility generates transgenic animals by pronuclear injections into F1 embryos. The facility generates targeted ES cells lines, which are used in blastocyst injections experiments to generate gene knock mice models.

X-RAY Crystallography



The facility provides state-of-the-art resources to elucidate 3-D structures of macromolecules and their complexes at atomic level. X-ray diffractometers are equipped with powerful microfocus rotating anode generators: (i) MicroMax™ 007 HF (Rigaku) Cu anode generator with Mar345-dtb image plate detector and (ii) FR-E+ SuperBright (Rigaku) dual wavelength Cu/Cr anode generators with R-axis IV++ image plate detector. 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 diffraction source is processed using crystallographic computational software. Molecular-modeling studies are performed using Intel Quad-Core windows and linux-based systems, Silicon Graphics (SGI-Fuel) workstations and software that are installed on CCMB server.

Small Angle X-ray Scattering (SAXS)

Facility is also equipped with in-house Small Angle X-ray Scattering System (S3-MICRO Point-Focus, Hecus X-ray systems, GmbH) for deciphering physical and structural features of macromolecules in solution. SAXS allows to probe size, shape, quaternary structure and complex formation of

biomolecules 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.

Dynamic light scattering-DLS (Nano-Biochem Technology-NaBiTech) is a useful tool to diagnose size distribution, stability, and aggregation state of macromolecules in solution prior to crystallization and SAXS studies.

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

High Throughput (HT) Crystallization Facility

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 protein dispensing and crystallization drop setting and (iii) Minstrel III along with two incubators (4°C and 20°C) automated for incubation, storage and inspection of plates for crystal growth. It is supported by 'on plate' UV imaging of crystals.

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

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 Standalone systems maintain lines for developmental biology, cell biology and behavioural biology studies. Facility also houses High end microscopy and imaging system (Model M205 FA) that has motorized advanced stereo fluorescence for multichannel fluorescence and bright-field imaging of zebrafish. Facility is equipped with Micro manipulation systems and trained staff to help researchers with genome editing technologies and to generate transgenic fishes. A computer aided tracking system (Danio vision with etho vision software) is available to carry research on behavioural aspects of the vertebrate model.

III.5 Research Resources

Instrumentation



CCMB has a strong and highly supportive Instrumentation group which takes care of the installation and maintenance of instruments. All the repair works are carried out in-house and no maintenance contracts are given. Maintenance of UPS, Video projection systems, the centralized Digital Imaging and Reprographic facilities 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 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 takes care of in-house design, development, modification and fabrication of instruments and also offers technical advice to other institutes in the 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.

Information Technology Group

The Information Technology group plays a major role in projecting the research and expertise of the lab to the external world, providing communication to



facilitate scientists to carry on research activities, collaborations, stay updated and connected with their counterparts around the world. The team coordinate with scientists in designing and building IT infrastructure required for R&D projects. It helps in framing IT strategy that supports the organizational objectives and optimised utilization of the resources. The team also develop software applications and tools to automate day to day activities in R&D management which helps to make decisions about strategic, financial and operational issues.

IT group associates in protecting IT infrastructure and research data against attacks from viruses and other threats. The team ensures right level of IT resources is available to meet changing levels of demand. It maintains and manages website, E-Mail, network and related services to support research activities. CCMB is connected to the leading Scientific and Technological Institutions, through an ultra high bandwidth 100 Mbps leased line connectivity from National Knowledge network and 8 Mbps leased line. LAN is established with 10 Gbps backbone, with switched 1 Gbps to desktops. Laboratory is also equipped with Modern Video conferencing facility. CCMB has also set up a HPC and Data centre for high-end computational requirements. The HPC is for analysis of large scale genomics datasets generated by Next-generation sequencing (NGS). Tools required for de novo genome assembly, analysis of ChIP-Sequencing and RNA Sequencing datasets, analysis of 3D chromatin organization data, etc were installed. Tools such as Gromacs are installed for Molecular Dynamics and simulations. Online applications for recruitments, Stores and purchase Indenting system, Automation of functioning of various facilities, Centralized Personnel record system, online ticketing systems, payroll, etc are developed by IT group as per the requirement.

A complete IP based Camera surveillance solution is installed at several locations in CCMB to elevate levels of security monitoring, management and enforcement. A centralized network access storage facility is created. All servers and user desktops are mounted with additional storage as per the requirement, to backup data. IT group provides training, so that staff can quickly make productive use of the new software or any new IT facilities. The team also provides ongoing support to users through a helpdesk on the intranet. For more details: <http://www.ccmb.res.in/itgroup/>

Fine Biochemicals Group



CCMB Fine-biochemicals facility stocks and maintains large number of biochemicals for the ongoing research activities of the laboratory. The facility has a Walk-in Freezer (-18°C) and a Cold Room in addition to two deep freezers (-20°C) for storage of chemicals as per the recommended conditions. The stocks of fine biochemicals include amino acids, proteins, enzymes, purification kits and buffer reagents.

In addition, stocks of restriction enzymes, antibodies, reagents necessary for purification and detection of recombinant proteins, reagents for DNA/protein gel electrophoresis, PCR, RT-PCR, DNA sequencing and synthesis and buffers, and gel electrophoresis are available. Chemicals related to proteomics and microarray analysis are also kept in the centralized facility. The requirement for these chemicals is monitored such that procurement is carried out on a regular basis, so as to maintain an uninterrupted supply. Requirement for these chemicals/enzymes is monitored with a help of software developed by CCMB IT Group. Availability of various chemicals can be seen by staff on CSIR-CCMB Intranet.

Library

CCMB shares a well-organized library with the Indian Institute of Chemical Technology (IICT). The library has an extensive collection of resource material in the fields of modern biology, chemistry, chemical engineering, and related areas, comprising 42,447 books, 61,985 bound volumes of periodicals and 1,85,000 Indian Patent Specifications. Full-text Indian and American Standards are available on CD-ROM. In addition to several volumes of classical journals dating back to the 19th century, complete sets of Science Citation Index (from 1945) and Chemical Abstracts (from 1907) are available. The library subscribes to 235 print journals and has

access over 4000 titles of online journals. In addition, CCMB has access to 80 e-Journals. A collection of PhD theses submitted by CCMB students is being maintained by assigning proper accession numbers since 2005. Haldane's book collection is also maintained by the Library staff. The library has an in-house bindery, reprographic facility, good Internet connectivity and a security surveillance system.

Experimental Farm, Horticulture and Landscaping

The Horticulture group maintains the lush green surroundings of the CCMB, LaCONES and CCMB Annexure-2. The newly developed experimental farm is being maintained by the staff of this group to meet the requirements of the plant genetic fingerprinting work. Different varieties of rice (indica, japonica), maize, as well as drought-resistant plants such as jojoba are grown in the farm. The Green House facility developed by this group is fully functional and is used for studies in plant molecular biology. Plants such as paddy, mustard and Arabidopsis are being grown here.

Rajbhasha Unit

This unit helps the Institution mainly in complying with various provisions of Official Language (OL) envisage by the GOI. For the past 17 years this unit is bringing out a popular science magazine

JIGYASA in Hindi dedicating every issue to a special topic of Life Sciences. This unit of Rajbhasha conducts every year "HINDI DAY" on 14th September and various Hindi competitions and programmes are organized as part of the occasion.

This year on 24th March the Rajbhasha unit has organized a KAVISAMMELAN EVEM MUSHAYARA by inviting famous Hindi & Urdu poets of Hyderabad. The Rajbhasha Unit has a very good library consisting 2600 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.

Engineering Services

CCMB Complex has a number of modern laboratories including the Annexe-1, LaCONES at Attapur and Medical biotechnology complex, Annexe-2, 2 Km away from the CCMB main campus. Facilities such as workshop, centralized air conditioning, class 10K clean rooms, bio-safety level III labs, ultralow freezers, walk-in cold rooms, walk-in freezer rooms, piped gas supply, pretreated water supply, liquid nitrogen, 3000Kva back-up generator sets, 33kv and 11Kv HT sub stations, distribution system, 30Kw solar power unit, etc., are being operated and maintained by the Engineering staff.



III.6 Services

A. Diagnostics

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. In the absence of specific treatment, molecular diagnosis, carrier detection, genetic counseling, pre-pregnancy monitoring, 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, etc. 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. In addition, the facility provides comprehensive education & training in application of molecular sciences for disease management.

Chromosome Diagnostics

Chromosomal abnormalities are implicated in mental retardation, congenital malformations, dysmorphic features, primary and secondary amenorrhea, reproductive wastage, infertility, disorders of sex development, neoplastic diseases. Cytogenetic evaluation of patients is helpful in the counselling and management of affected individuals and families. Prenatal diagnosis of chromosomal abnormalities in high-risk pregnancies helps in detecting chromosomally abnormal fetuses. 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.

B. Laboratory for the Conservation of Endangered Species (LaCONES): Facilities & services

LaCONES, CSIR-CCMB Annexe 1, is situated in Attapur, Rajendernagar, Hyderabad. LaCONES provides extensive service to the nation for wildlife



diagnostics. The different types of services provided at LaCONES are:

- Species identification
- Individual identification/ relatedness/paternity testing
- Sexing of birds
- PCR based diagnosis (RTPCR) for RNA/DNA virus related diseases
- Diagnosis of *Batrachochytrium dendrobatidis* (BD) using nested PCR and qPCR
- Fertility (Progesterone/Estradiol/Testosterone) including pregnancy detection and stress hormone (Cortisol) analysis
- Semen and Sperm profiling with/without chemical immobilization

Wildlife diagnostics

LaCONES is playing an important role in generating meaningful DNA evidence for law enforcement agencies to unambiguously identify species targeted in poaching and illegal wildlife trade since 2000. The DNA technique developed and patented by CCMB allows identification of a biological specimen of unknown origin and delineates its utility to the level of family, genus and species. Biological specimens are received from all over the country confiscated and forwarded by state forest, judiciary, police and custom departments. During the period (2000–till August 2017) a total of 1554 wildlife crime cases were solved at LaCONES, which includes more than 3492 biological samples. This effort has also significantly helped in increasing the awareness amongst the forest and police officials regarding the usefulness of this technique and also in inculcating a sense of fear amongst the poachers. In addition, LaCONES is also providing genetic health check-up, disease diagnosis and reproductive hormones monitoring services to various zoological parks in the country under Conservation Breeding program of Central Zoo Authority of India for 25 endangered species. More than 100 reports have been submitted to different zoo in the period 2007-2017.

Cryo-banking

Cryo-preservation of semen of endangered species is an ongoing activity in LaCONES and cryopreservation protocols have been optimized for semen from lions, tigers, leopards, spotted deer and black buck, blue rock pigeon and white-backed

vultures. These studies indicated that the protocol for cryopreservation is species specific and needs to be standardized for and each every species. The cryo-preserved semen samples could be used for AI under circumstances when a male factor is involved in normal mating could be used to produce embryos by in vitro fertilization. In contrast to semen, non-availability of oocytes of wild animals is a major challenge in cryopreservation of the female gamete. Though electro-ejaculation is permitted for semen collection, laparoscopic intervention is not permitted for oocyte collection. An alternative approach would be to recover oocytes from the ovaries at postmortem activate them, mature them and store them. LaCONES has carried out pioneering work in this area and has succeeded in oocyte recovery, cryopreservation, IVM and IVF of oocytes collected post-mortem from the ovaries of black buck, nilgai and chousingha at the Nehru Zoological Park (NZN), Hyderabad. In addition, attempts could be made to establish species-specific protocols for cryo-preservation of ovaries and testes from dead wild/endangered animals. Cryo-banking of genes, tissues, gametes, ovaries, testes and embryos provides a holistic approach towards preservation of the germplasm from endangered animals. These cryo-preserved bioresources could over the years be used for comparing genetic polymorphism with the existing populations and also used for somatic cell nuclear transfer or inter-species nuclear transfer, thus satisfying the long term goal of LaCONES in wildlife conservation.

Lectures, Training & workshops

LaCONES also works towards generating awareness/creating skilled resources through regular lectures, in-house hand's on trainings/workshops about the importance and usage of molecular approaches towards wildlife conservation for dissertation students (M. Sc., B. Tech. & M. Tech), students of National Institute of Criminology and Forensic Science, Delhi, Officers and Probationers of Telangana/AP Forest Academy, Central Forensic Science Laboratory, Indian Forest Academy and National Police Academy. The scientists also visit forest head quarters/forest academies to impart knowledge about DNA and other techniques and demonstrate sample collection, storage & transportation to the lab.

III.7 CCMB Innovation Hub



From Left to Right: Voleti Sreenivas, V R Sundareeswaran, Jyotsna Dhawan, N Madhusudhan Rao, CB Tripura Sundari, B Kiran kumar, K Anitha (Top row) YSSV Prasad, PS Raju, Anuradha

Innovation HUB (iHUB) is a new initiative to bring together the extension activities of CCMB. These activities include taking the research leads to the next step of translation, promoting the skill development programs and enhancing the ongoing diagnostic activity. By grouping these activities and by creating a section 8 company by name ASPIRE (Association for Promoting Innovation and Research Entrepreneurs (CIN U73100TG2017NPL117299), iHUB would grow into a division that interacts with the industry and other stakeholders to develop business plans for inventions at CCMB, training programs etc. iHUB also manages and promotes the objectives of CRTDH, a facility hub created by DSIR to cater to the needs of the small and medium enterprises. iHUB conducts its activities from CCMB Annexe 2.

A small brief of each of the sub-activities is as follows:

Innovation Cell: A technology advisory group (TAG) identifies leads of CCMB that have potential commercial application. To reduce the load on the PI on the lead, the TAG team prepares a plan to take the leads forward in such a way so as to make them attractive to the industry and also identifies proteins that can be taken up for in-house production.

CRTDH: Innovation is the key for successful entrepreneurship. Many companies are unable to translate new technologies and ideas into marketable products and services posed by innovation challenges such as space, infrastructural and R & D constraints. CRTDH was established with the help of DSIR and started to function from January 2016. The facility has essential equipment for conducting projects in biology.

Till date, we have five start-ups working in our facility-

1. Oncosimis Biotech Pvt Ltd., 2. Kommareddy Biopharma Pvt Ltd. 3. Virupaksha Life Sciences Pvt Ltd., 4. Bioartists Pvt Limited., 5. Theranosis Life Sciences Pvt. Ltd.

Our Start - UPS:

Bioartists Life Sciences Pvt Ltd.,

Oncosimis Biotech Pvt Ltd.,

Kommareddy Biopharma Pvt Ltd.

Virupaksha Life Sciences Pvt Ltd.,

Theranosis Life Sciences Pvt. Ltd.

iHUB also facilitates execution of external requests for various techniques and equipment. Companies that have availed this facility so far are: Stelis Biopharma Pvt Ltd., Bangalore, Suven Life Sciences Pvt Ltd., Hyderabad, Mylan laboratories, Hyderabad, Gland Pharma, Hyderabad, Genes and Life Pvt Limited, Hyderabad, Nuziveedu Seeds, Hyderabad, Apollo Hospitals, Hyderabad, Biophore India Pharmaceuticals Pvt Ltd., Hyderabad.

Atal Incubation Center

CSIR-CCMB as part of its incubation and translational activity has established an incubation centre at its Annexe2. With the experience of the incubator, recently CCMB has applied for a grant managed by Atal Innovation Mission (AIM) under NITI Aayog. The mission is aimed at promoting a culture of innovation and entrepreneurship in the country. It aims to create high-class incubation facilities with necessary infrastructure in terms of capital equipment and operating facilities, coupled with the availability of sectoral experts for mentoring the start-ups. The incubation centres would support and encourage start-ups in the health sector. NITI Aayog has chosen the Centre for Cellular and Molecular Biology (CCMB) for hosting the ₹10-crore Atal Incubation Centre aimed at identifying promising start-ups, creating facilities and guiding them to success in the field of biotechnology. CCMB has formed a section 8 company to govern the affairs of the center and has entered into a licence agreement with three companies involved in biotechnology. These companies would conduct their translational activities from the new incubator.

IV. Administration and Management

Director's Office



From Left to Right: MR Vasanth Kumar, S Madhuri, D Lavanya, Rakesh K Mishra, RV Subba Rao, BVN Naveen Kumar, G Satyanarayana

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 and Accounts

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



Stores and Purchase

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 which is supervised by trained security officers on the staff of the CCMB.



KVVS Raveendra kumar and CV Tirumala Rao

Medical Services

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



Left to Right: T Nagalaxmi, UV Sitaramamma, Dr G Sujatha, Dr R Palnitkar, M R Ravindernath, A Mahesh, D Ravinder Reddy, G Tirupathiah, G Vinod kumar

Guest House Staff

CCMB 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 (PME)

The primary responsibility of this group is to assist the Director CCMB, in all activities of CCMB and act as a liaison between the Director and the other research groups, CSIR-HQ and other organisations. The PME unit takes care of various in-house, sponsor, collaborative, grant-in-aid, and NIMTLI projects and provides inputs in the preparation of project proposals and other documents and reports of the CCMB. It organizes National and International visits of Presidents, Ministers, Parliamentarians and Science delegations to the Centre. This group co-ordinates organising national and international seminars, symposia, workshops, conferences and training programs and maintains contacts with all CSIR

departments in obtaining sanctions and required approvals for this activity. In addition it coordinates periodical audit sessions on CCMB by CSIR audit teams. PME takes up Diagnostic services and coordinates popular school visits. The team also participates in conferences, exhibitions to showcase CCMB technologies in coordination with CSIR-HQ. This group also coordinates data for sending RTI replies.

During the year, the group has developed PROJECT is, a project IT module, with the help of the IT division of CCMB by providing all inputs and data relevant to the maintenance of projects. The module contains details of ongoing projects in a user-friendly format with a provision of obtaining status reports of any in-house project at a given time including financials.



From Left to Right: P Satguru, MV Subbarao, PS Raju, Gulzar Khan, MR Vishnu Priya, K Anitha, K Satyanarayana, BV Ramakrishna

Business Development Unit

This unit interacts with sponsors in identifying their needs and generating project proposals. It also participates in conferences, exhibitions to showcase CCMB technologies and coordinates with the CSIR in filling and granting of patents.

Publications and Public Relations

The main responsibility of this unit is to make the general public aware of the latest developments of

the Centre. Information about CCMB research is conveyed to public via print and electronic media, publications such as Annual Report and through other brochures. Press releases describing CCMB's new initiatives, innovations, scientific leads and path-breaking results are given to both national and local level newspapers. During the year, several press meets were organized to explain the major contributions of CCMB to the electronic media by CCMB scientists.

A network graph with nodes labeled with codes like JP09, KR10, IN55, etc. Node B is a central hub with many connections. Node J is highlighted with a dashed arrow. A red oval highlights a cluster of nodes including JP09, JP10, and IN55. Two yellow and green leaf-like shapes frame the central text.

V. General Information

Research Council and Management Council

A. 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.

Dr Sandip K Basu Professor of Eminence National Institute of Immunology New Delhi	Chairman	Dr Rajesh Gokhale Director Institute of Genomics and Integrative Biology Delhi	Member
Prof Vidyanand Nanjundiah Centre for Human Genetics Bangalore	Member	Prof Ram Rajasekharan Director CSIR-Central Food Technological Research Institute Mysore	Member
Dr Dinakar M Salunke Executive Director Regional Centre for Biotechnology Gurgaon	Member	Prof Siddhartha Roy Senior Professor & Dean of Studies Bose Institute Kolkata	Member
Prof J P Khurana Professor Department of Plant Molecular Biology University of Delhi South Campus New Delhi	Member	Dr Rakesh K Mishra Director Centre for Cellular & Molecular Biology Hyderabad	Member
Prof R Sukumar Centre for Ecological Sciences Indian Institute of Science Bengaluru	Member	Dr Sudeep Kumar Head, Mission Directorate CSIR New Delhi 110 001	Member
Dr Krishna Ella Chairman and Managing Director Bharat Biotech International Limited Hyderabad	Member	Shri A V Chainulu Scientist F DSIR, Technology Bhawan New Delhi	Member
Dr T S Rao Adviser Department of Biotechnology New Delhi	Member	Dr K Thangaraj Senior Principal Scientist Centre for Cellular & Molecular Biology Hyderabad	Secretary

Research Council and Management Council

B. Management Council

Following is the composition of the Management Council of CSIR-CCMB for the period 01.07.2016 to 31.12.2017 as approved under Rule-65 of the CSIR Rules & Regulations:

Dr. Rakesh K Mishra Director, CSIR-CCMB Uppal Road Hyderabad-500007	Chairman	Dr. Ramesh V. Sonti Chief Scientist CSIR-CCMB Hyderabad	Member
Dr. Bokara Kiran Kumar Scientist CSIR-CCMB Hyderabad	Member	Dr. A.S. Sreedhar Principal Scientist CSIR-CCMB Hyderabad	Member
Dr. H.H. Krishnan Senior Scientist CSIR-CCMB Hyderabad	Member	Shri Y V Rama Rao Principal Technical Officer CSIR-CCMB Hyderabad	Member
Dr. V.M. Tiwari Director CSIR-NGRI Hyderabad-500007	Member	Prof. R K Sinha Director CSIR-IMTECH Chandigarh-160036	Member
Dr. M R Vishnu Priya Head, PME, CSIR-CCMB Hyderabad	Member	Finance & Accounts Officer CSIR-CCMB Hyderabad	Member
Controller of Administration CSIR-CCMB Hyderabad	Member-Secretary		

Ongoing MOUs & Agreements

- Royal Melbourne Institute of Technology, Melbourne, Australia
- DUPONT India Pvt. Ltd. Hyderabad
- Acharya N.G. Ranga Agrl. University, Hyderabad
- King Edward Memorial Hospital Research Centre, Pune
- Directorate of Rice Research, Hyderabad and Metahelix Life Sciences, Hyderabad
- MNR Dental College & Hospital, Medak District
- NIMHANS, Bangalore
- Madras Diabetes Research Foundation, Chennai
- Sunshine Medical Academy of Research & Training, Hyderabad
- Directorate of Rice Research & Agri Biotech. Foundation, Hyderabad
- Deccan College Post Graduate and Research Institute, Pune
- All India Institute of Medical Sciences, Delhi
- L.V. Prasad Eye Institute, Hyderabad
- Sickle Cell Institute Chhattisgarh, Raipur
- Nizam's Institute of Medical Sciences, Hyderabad
- Administrative Staff College of India
- Indian Institute of Rice Research (IIRR), Hyderabad
- National Academy of Agricultural Extension Management (MANAGE) Hyderabad
- Telangana State Director of Medical Education
- B.E. Ltd
- IncuMed, Hyderabad
- CCMB-IMMT-Genomix Biotech Inc. USA

CRTDH MOUs and License Agreements

- NIMS, Hyderabad
- Theranos Life Sciences Pvt. Ltd, Hyderabad
- National Research Development Corporation (NRDC), New Delhi
- Virupaksha Lifesciences Pvt. Ltd. Hyderabad
- Bioartis Life Sciences Pvt. Ltd., Hyderabad
- Oncosimis Biotech. Pvt. Ltd.
- IKP Knowledge Park
- Kommareddi Biopharma Private Limited
- DME, Govt of Telangana
- iBUILD Innovations India Ltd.
- Bioserve Biotechnologies (India) Pvt. Ltd.

Atal Incubation Centre-CCMB CRTDH Agreement

- Magellan Life Sciences Private Limited, Hyderabad
- Lycan Lab, Bangalore
- SAATRA Capital Advisors LLP

Visitors to CCMB & SEMINARS

The national and international linkages forged by the CCMB resulted in drawing a large number of scientists, experts, consultants and applicants in the frontier areas of cellular and molecular biology to visit and deliver lectures at the Centre. The list of such seminars organized during the year is given below:

Dr Pradeep Kumar Burma,

Department of Genetics,
University of Delhi South Campus, New Delhi
"Developing insect resistant Bt cotton lines - our experience" April 04,2016

Dr Manikandan Narayanan,

National Institutes of Health,
Bethesda, MD, USA
"Gene networks in health and disease: a computational systems approach" April 18,2016

Dr Suhel Quader,

Scientist - Nature Conservation Foundation &
Head - Citizen Science Programmes at NCBS,
Bangalore
"Citizen Science: a powerful approach to ecological research and monitoring" April 19,2016

Dr Samarendra K Singh,

University of Virginia, Charlottesville, Virginia,
USA
"Regulation of Cell Cycle and Replication in Cancer Cells" May 10,2016

Nathan Ikaika Joel Naktsuka,

Harvard Medical School, Boston, USA
"Admixture Mapping in African-Americans Uncovers Variants Explaining the Difference in Prevalence of Multiple Sclerosis in Europeans Compared to Africans" June 03,2016

Dr Jairam K.P. Vanamala,

Center for Molecular Immunology & Infectious Diseases, Penn State University, USA
"Food-Approach to Target Cancer Stem Cells" June 14,2016

Dr Anita Malhotra,

Bangor University, UK
"Anolis lizards: Coping with climate change" June 14,2016

Dr Wolfgang Wuster,

Bangor University, UK
"Why is snake venom composition so variable?" June 15, 2016

Dr Sambashiva Banala,

Howard Hughes Medical Institute, Ashburn, VA, USA.
"Development of Novel Chemical Tools for Biological Applications" June 27,2016

Dr Arni S.R.Srinivasa Rao,

Dept of Biostatistics & Epidemiology, Augusta University, USA
"Modeling movement of poultry birds for understanding parasite spread" June 29,2016

Dr Thirumala-Devi Kanneganti,

St. Jude Children's Research Hospital, Memphis, USA
"Regulators of Inflammatory Responses" June 30,2016

Dr Gopinath Meenakshisundaram,

Laboratory of Translation Control of Disease, Institute of Medical Biology, Singapore.
"Can injury cause cancer? Epithelial carcinomas hijack a molecular switch from wound healing to promote their invasion" July 04,2016

Dr Tofajjen Hossain Sk,

University of Miami, Miami, FL, USA
"Breaking Down Barriers: How RNase R Degrades Structured RNA" July 18,2016

Dr Regalla Kumarswamy,

Hanover Medical School, Hannover, Germany
"Functional non-coding RNAs in patho-physiology of the heart" July 28,2016

Dr Jameel Ahmad Khan,

Lifecode Technologies Pvt. Ltd. New Delhi
"Good Laboratory Practices (GLP) in Cell Culture lab: Cell Line Authentication and Cross-Contamination" August 05,2016

Dr Ashish Arora,

Molecular and Structural Biology Division, CSIR-Central Drug Research Institute, Lucknow
"Unraveling the stereochemical and dynamic aspects of the catalytic site of bacterial peptidyl-tRNA hydrolase" August 09,2016

- Dr S Subramanian, Dept of Surgery,**
University of Minnesota, USA
"Mechanisms of Malignant Transformation and Immune Response in Colon Cancer"
August 16, 2016
- Dr P Suresh,**
National Animal Resource Facility, NIN, Hyderabad
"CPCSEA SOP for IAEC - Role and responsibilities in animal experimentation" August 17, 2016
- Prof Geeta K Vemuganti,**
School of Medical Sciences, University of Hyderabad, Hyderabad
"Regulatory approvals for conducting Stem Cell Research in India: an overview" August 17, 2016
- Dr Aruni Wilson,**
Division of Microbiology and Molecular Genetics, School of Medicine, Loma Linda University, USA
"Novel Microbial factors leading to microbiome dysbiosis predisposing to systemic diseases "
August 29, 2016
- Dr Shankar Srinivas,**
Department of Physiology Anatomy & Genetics, University of Oxford, UK.
"Development of form and function of the embryonic heart" August 29, 2016
- Dr Marilyn J. Telen,**
Duke University Medical Center, Durham, North Carolina, USA
"Outcome Modifying Genes in Sickle Cell Disease and Outlook for New Pharmacologic Therapeutic Targets" September 14, 2016
- Dr Dhanasekaran Shanmugam,**
Biochemical Sciences Division National Chemical Laboratory, Pune
"Metabolic plasticity dictates fitness and differentiation in *Toxoplasma gondii* and reveals potential targets for therapeutic intervention" September 22, 2016
- Prof L S Shashidhara,**
Indian Institute of Science, Education & Research (IISER), Pune
"Growth control: lessons from *Drosophila* for cancer biology" October 05, 2016
- Dr Suchitra Gopinath**
Pediatric Biology Center, THSTI, Faridabad
"Vitamin D signaling in skeletal muscle growth and atrophy" October 19, 2016
- Dr Randal Halfmann,**
Stowers Institute for Medical Research, Kansas City, USA
"Quantitative Prion Biology" October 20, 2016
- Dr Chetana Sachidanandan,**
IGIB New Delhi
"Modeling human disease in zebrafish" October 20, 2016
- Dr Ramkumar Sambasivan,**
InStem, Bengaluru
"Mesoderm patterning and early vertebrate development" October 20, 2016
- Dr Gireesha Mohannath,**
Departments of Biology, Molecular and Cellular Biochemistry, Indiana University, USA
"New hypothesis: chromosomal context determines activity status of rRNA genes on a multimegabase scale in *Arabidopsis*" October 28, 2016
- Dr Srimonta Gayen,**
Department of Human Genetics, University of Michigan Medical School, USA
"Epigenetic regulation by long non-coding RNAs and histone modifiers through the lens of X-chromosome inactivation" November 03, 2016
- Dr Ratna Ghosal,**
Minnesota Aquatic Invasive Species Research Center, University of Minnesota, USA.
"From endangered to invasive species: hormone-pheromone-behavior interactions in conservation" November 10, 2016
- Dr Venkata R Chalamcharla,**
National Cancer Institute, NIH, USA
"Transcription termination primes RNA-mediated epigenetic genome control" November 16, 2016
- Prof. Rekha Samuel,**
MC Vellore
"Pathophysiology and Molecular Pathways Regulating Pericyte Phenotype in Type 2 Diabetes. The Gestational Diabetes Mellitus Placental Model." November 17, 2016
- Dr Kartik Sunagar,**
Hebrew University of Jerusalem, Israel
"Deadly innovations. 'Venomics' for evolution, ecology, and snakebite management" November 18, 2016

- Dr Shekhar Kumar Niraj, IFS,**
TRAFFIC - India at World Wide Fund for Nature,
Delhi
"Illegal Wildlife Trade Scenarios in India and South Asia" November 22,2016
- Dr Santosh Kumar,**
Stanford University, USA
"Molecular Events of Lymphocyte Recognition and Response" December 02,2016
- Dr Dipankar Bhandari,**
Max Planck Institute for Developmental Biology,
Tubingen, Germany
"Role of the CCR4-NOT deadenylase complex in the post-transcriptional gene silencing" December 14,2016
- Dr Sergey Naidenko, A.N.**
Severtsov Institute of Ecology and Evolution,
Moscow, Russia
"Behavioral Ecology of carnivores in Russia"
December 19,2016
- Dr John Waitumbi,**
United States Army Medical Research Directorate
(USAMRU), Nairobi, Kenya
"Effects of Gender on the Intra-erythrocytic concentration of the Malaria Protective deoxy-hemoglobin S and Interactions of alpha-thal and G6PD Genotypes on Malaria in Children Living in the Lake Victoria Basin, Kenya"
December 20, 2016
- Prof James Bradley,**
University of Liverpool, England
"Plasma discharges for the ambient processing and detection of materials" December 21, 2016
- Dr Ganesh Bagler,**
IIIT, Delhi
"Data-driven discovery of the molecular essence of Indian cuisine" December 23,2016
- Dr Kaustuv Sanyal,**
JNCASR, Bangalore
"Genome indexing by histone H3 variants in *Candida albicans*" December 27,2016
- Dr Bhavana Muralidharan,**
Dept of Biological Sciences, TIFR, Mumbai
"Molecular mechanisms regulating cell fate specification in the developing cerebral cortex"
January 12,2017
- Dr Dhananjay Huilgol**
"Developmental mechanisms of projection neurons in the forebrain" January 12, 2017.
- Prof Aseem Ansari,**
The Genome Center of Wisconsin,
Department of Biochemistry, University of Wisconsin-Madison, USA
"New writers and old readers of under-explored phosphorylation marks on the tail of RNA polymerase II" January 17, 2017
- Dr Sundar Balasubramanian,**
Medical University of South Carolina, Charleston, USA
"A Journey from Pranayama to Biomarkers"
January 24,2017
- Dr Dibyendu Bhattacharyya,**
Advanced Centre for Treatment, Research and Education in Cancer (ACTREC),
Tata Memorial Centre, Navi Mumbai
"Size, Shape and Dynamics of Golgi and ER"
January 24,2017
- Dr Robert J. Freishtat,**
George Washington University School of Medicine and Health Sciences, Washington, USA
"Adipocyte-derived exosomes: a novel mechanism for obesity-related disease" January 25,2017
- Dr Jose Sebastian,**
Carnegie Institution for Science, Stanford University, USA
"Dealing with Stress: Cereal roots enact austerity measures during drought to bank water" February 06,2017
- Dr Jayasri Das Sarma,**
Department of Biological Science, IISER, Kolkata
"Gliopathy are early events in viral induced CNS demyelination" February 15,2017
- Prof Andrew Clayton,**
Swinburne University of Technology, Melbourne, Australia
"Can fluorescence help us to understand the structure of cell-surface molecules?" February 20,2017
- Prof Jean-Marie Ruyschaert,**
Universite Libre de Bruxelles, Brussels, Belgium
"Protein and lipid nanoparticles activate the immune system through recognition of Toll-like receptors" February 21,2017
- Dr Nigel M. Hooper,**
University of Manchester, Manchester, U.K.
"Lipid Rafts in Prion Biology and Alzheimer's Disease" March 06,2017

Dr Bruce A. Shapiro,

RNA Biology Laboratory,
National Cancer Institute, NIH, Frederick,
Maryland, U.S.A.
"Understanding RNA Structure and Function:
Prediction, Analysis and Nanodesign" March
07,2017

Dr Alok K Sinha,

National Institute of Plant Genome Research, New
Delhi
"Submergence Tolerance in Rice: A new twist in
regulation of SUB1A1 by a mitogen activated
protein kinase" March 09,2017

Dr Murali Krishna Cherukuri,

National Cancer Institute, NIH, Bethesda, USA
"Metabolic MRI: Preclinical and Clinical
applications in Cancer Imaging" March 17,2017

Dr Somenath Bakshi,

Systems Biology Laboratory, Harvard University,
USA
"Single-cell Measurement of Microbial Stress-
response Dynamics in Complex Growth
conditions" March 17,2017

Dr Prasad Kasturi,

Max-Planck Institute for Biochemistry, Munich,
Germany
"Protein homeostasis during stress and aging in
C.elegans" March 20,2017

Dr Ritesh Kumar,

Texas A&M, Health Science Centre, Houston,
Texas, USA
"Microbes meet cancer: Role of *Streptococcus*
gallolyticus in colorectal tumor development"
March 21,2017

Dr Saikat Chowdhury,

Dept. of Integrative Structural & Computational
Biology,
The Scripps Research Institute, La Jolla, USA
"Visualizing dynamic cellular machinery with
electron microscopy" March 30,2017

Dr Gyaneshwer Chaubey,

Department of Evolutionary Biology, Estonian
Biocentre, Tartu
"Peopling of South Asia: Genesis and
Implications" March 31,2017

Visits to CCMB by students/faculty/experts during 2016-17

CCMB encourages visitors from different parts of the country and abroad. During the year 2016-17, the total visits were 60 and the visitors were 1913. Some of the visitors during 2016-17 are as follows:

- Ahmediya International School, Mauritius
- Wisconsin College, USA
- Science INDIA Forum, Bahrain
- Edinberg University Professors, UK
- Kendriya Vidyalaya AFS, Hyderabad
- ASCI, Hyderabad
- Telangana Tribal Welfare Society, All districts of Telangana
- College Of Veterinary Science, Gujarat
- Bhavans College, Mumbai
- Fergussons College, Pune
- Indian Institute of Rice Research, Hyderabad
- University of Kashmir, Srinagar
- CMS College of Science & Commerce, Coimbatore
- Nizams Institute of Medical Sciences, Hyderabad
- Shivaji University, Kohlapur
- KIIT University, Bhubaneswar
- Loyola College, Hyderabad
- Savithribai phule University, Pune
- Tamilnadu Agricultural University, Tamilnadu
- Nagindas Khandwala college,
- Jain University, Bangalore
- Sree Sankara College, Kalady, Ernakulam
- Abhyasa School, Medak
- St.Xavier college, Kerala
- Sathaye college, Mumbai
- Davangere University, Karnataka
- National Institute of Nutrition(NIN), Hyderabad
- MES Mampad college, Kerala
- St.Joseph college, kerala
- Govt. Degree college, Siddipet
- University of North Bengal, West Bengal
- Sage School, Hyderabad
- Kerala Veterinary & Animal Sciences University, Mannuthy
- Orissa University of Agriculture and Technology, Bhubaneswar
- PSGR Krishnammal college for women, Coimbatore
- LV Prasad Eye Institute, Hyderabad



Introduction on CCMB to students

Apart from the student visits, CCMB organised special programs for the students.

- CCMB-Young Innovators Programme [YIP]: YIP is a programme for the school children of classes VIII to X, provides the students an opportunity to have

close interactions with the CCMB scientists and also provides hands on experience of research work for a period of two weeks from 9th to 20th January 2017 to 24 students from various schools selected after written test.



Young Innovators interacting with researchers



Young Innovators with Director, CCMB

The precursor events of IISF 2016 at CSIR-CCMB, Hyderabad from November 01-15, 2016:

CSIR-Centre for Cellular and Molecular Biology, Hyderabad planned and executed several programmes from November 1 to 15, 2016 as a part of India International Science Festival (IISF-2016), under the Ministry of Science and technology and Ministry of Earth sciences in association with Vigyana Bharati (VIBHA). The event involved visit to CCMB by many students, faculty and public.



Honours & Awards

Institutional Honours

An inter-institutional team of researchers from CCMB and the Indian Institute of Rice Research (formerly Directorate of Rice Research) have received the **BIOTECH PRODUCT PROCESS DEVELOPMENT AND COMMERCIALIZATION AWARD-2016** awarded by Department of Biotechnology, Government of India.

A. Research staff

Amitabha Chattopadhyay

- * Awarded The World Academy of Sciences (TWAS) Prize in Biology
- * Elected as Associate Editor, Molecular Membrane Biology
- * Elected as Member, Wellcome Trust DBT India Alliance's Early Career Fellowship Selection Committee
- * Invited to be Discussion Leader, Gordon Research Conference on Bio interface Science: Active, Adaptive, and Responsive Bio interfaces, Les Diablerets, Switzerland
- * Invited to give Keynote Lecture at the IUBMB/IUPAB/IUPS Joint Advanced School on Receptors and Signaling, Spetses island, Greece
- * Invited to give 74th CSIR Foundation Day Lecture, Central Drug Research Institute, Lucknow
- * Invited to give Mentor Lecture at the Science Communication Workshop, DBT-
- * Wellcome Trust India Alliance, Hyderabad

Ch. Mohan Rao

Life Time Achievement Award (Felicitation by the Chief Minister of AP, on the occasion of AP Science Congress, Andhra Pradesh Akademi of Sciences, Tirupati) (2016)

Adjunct Professor, RMIT University, Melbourne, Australia (2016-2019)

Honorary Visiting Professor, University of Mysore (2016-)

Adjunct Professor, Dept of Microbiology, Mangalore University, Mangalore (2016-

Invited talks and conference presentations during the year:

- * IUPAB Council meeting, The 19th International Union of Pure and Applied Biophysics (IUPAB) and 11th European Biophysical Societies' Association (EBSA) Congress, Edinburgh, Scotland, 16-10 July, 2017

- * "Clusterin: full-length protein and one of its chains show opposing effects on cellular lipid accumulation" 5th Conference of the Asia Pacific Protein Association and the 12th International Symposium of the Protein Society of Thailand (5th APPA/12th PST joint conference), Bangsaen, Thailand, 11-14 July, 2017.
- * 27th The World Academy of Sciences, General Meeting, Kigali, Rwanda, 12-17 November, 2016.

Rajan Sankaranarayanan

Dr. M. R. Das Memorial lecture, 2017 awarded by INSA, New Delhi

Yogendra Sharma

Research featured on the Cover Page of American Chemical Society journal Biochemistry, March 7, 2017 issue (<http://pubs.acs.org/toc/bichaw/current>).

Jyotsna Dhawan

- * Member, Scientific Advisory Committee, World without GNE Myopathy
- * Member, Board of Governors, Trans-Disciplinary University, Bengaluru
- * Co-Organizer, International Congress of Cell Biology 2018
- * DBT-Danish Research Foundation grant for Indo-Danish Collaborative Center for Musculoskeletal Stem Cells and Regeneration (MUSTER)

Purnima Bhargava

Invited as Session Chair and Speaker at "The 10th International Conference on RNA polymerases I, III, IV and V", University of Michigan, Ann Arbor, USA from June 24th-27th, 2016.

Mandar Deshmukh

Invited speaker at 7th Asia Pacific NMR Symposium, Bangalore, 02/2017.

Promoted speaker at 27th ICMRBS (International Conference on Magnetic Resonance in Biological Systems) held at Kyoto, Japan 08/2016.

Amit Asthana

Received Award of Honor, as Distinguished Speaker at Lab-on-a-Chip and Microfluidic Conference, Organized by Select Bio India, 26th-27th May 2016, Bangalore, India.

Regalla Kumaraswamy

Wellcome Trust/ DBT India Alliance Intermediate Fellowship

Lekha Dinesh Kumar

- * Selected as the external expert for DME Telangana MERIT program.
- * Editor of Springer protocols Molecular Biology Series; RNA interference and cancer therapy.

K. Lakshmi Rao

Elected as Fellow of Andhra Pradesh Academy of Sciences (FAPAS), Amravathi, AP, India 2016

V. Vijaybhaskar

Selected as Editorial board member of International Journal of Applied Agricultural Sciences (IJAAS), Science Publishing Group, New York, USA.

Ira Bhatnagar

Received International Association of Advanced Materials Scientist Medal (IAAM Scientist Medal) for the year 2017 from the International Association of Advanced Materials at the International Conference on Nanomaterials and Nanotechnology 2017, 01st March 2017.

Manjula Reddy

- * Invited speaker at EMBO conference on 'Bacterial cell division: orchestrating the ring cycle' held at Prague, Czech Republic from 14-17 September 2016
- * Invited speaker at EMBO conference on 'Bacterial Morphogenesis, Survival and Virulence: Regulation in 4D' held at Trivandrum, India from 27-30 November 2016

Umapathy

- * Delivered a talk and participated in the Science Camp, sponsored by the Department of Science and Technology (DST), Government of India as part of its INSPIRE program, at SKR & SKR Govt. College for Women, Kadapa, Andhra Pradesh from 20th Oct 2016 to 24th Oct 2016
- * Delivered a talk and participated in the Science Camp, sponsored by the Department of Science and Technology (DST), Government of India as part of its INSPIRE program, at LOYOLA DEGREE & PG COLLEGE (YSRR), PULIVENDLA, Kadapa(dt), Andhra Pradesh from 29th November to 3rd December 2016.

G. Mala

Received Travel Grant from DBT - INDIA and CSIR to attend and present a poster at the 84th European

Atherosclerosis Society Congress at INNSBRUCK--AUSTRIA held between May 29th-June 1st-2016.

Sanjeev Chavan Nayak

Award of National Overseas Scholarship, Under the Scheme of National Overseas scholarship for ST students, Government of India, Ministry of Tribal Affairs.

B. Awards to PhD students and other staff members

Anand Kumar Sharma

- * Awarded "National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Scholarship" from Keystone Symposia meeting New Therapeutics for Diabetes and Obesity (G1), held at Estancia La Jolla Hotel & Spa, La Jolla, California, USA April 17-20, 2016.
- * Selected for the Travel award for oral/poster presentation in "Neuronal Calcium Sensors in health and disease" Meeting, Germany, December 3-7, 2016.

Shanti Swaroop Srivastava

2017 Ratna Phadke Award of Indian Biophysical Society (IBS), Annual Meeting at IISER, Mohali.

Radhika Khandelwal

- * Selected for the Travel award for oral/poster presentation in "Neuronal Calcium Sensors in health and disease" Meeting, Germany, December 3-7, 2016.
- * Received Carl Storm International Diversity (CSID) fellowship from Gordon Research Conference (GRC) to attend GRC-Calcium Signaling Conference at Renaissance Tuscany II Ciocco Lucca (Barga), Italy, 2017.

M. Jafurulla

Awarded IUBMB/IUPAB/IUPS fellowship to participate in IUBMB/IUPAB/IUPS Joint Advanced School on Receptors and Signaling, Spetses island, Greece.

Parijat Sarkar

Awarded FEBS Youth Travel Fund to participate in FEBS advanced course on Lipid-protein interactions and organelle function, Spetses island, Greece.

Debarya Saha

Travel Award for Poster Presentation at Annual Review of Research 2017 (inStem).

Pratibha Bhalla

- * Recipient of EMBO travel grant for “Gene transcription in yeast: from chromatin to RNA and back” conference held at Sant Feliu de Guixols, Spain (June 11-16, 2016).
- * Recipient of EMBL travel grant for “Transcription and Chromatin” conference held at EMBL, Heidelberg, Germany (Aug 27-31, 2016)

Ashutosh Shukla

- * Recipient of EMBO travel grant for “Chromatin and Epigenetics” conference held at EMBL, Heidelberg, Germany (May 3-6, 2017).
- * Best poster presentation award in “11th Asian

Epigenomics meeting”, held at JNCASR, Bengaluru, India (September 30-October 1, 2016).

- * Recipient of EMBO travel grant for “Gene transcription in yeast: from chromatin to RNA and back” conference held at Sant Feliu de Guixols, Spain (June 11-16, 2016).

Prabhavathy Devan

Awarded DST National PDF fellowship

Renu Sudhakar

Received Best POSTER AWARD at the "Malaria Parasite Biology: Drug Designing & Vaccine Development" conference at Nirma University, Ahmedabad, September 9-10, 2016.

Participation in International Meetings, Courses and Deputations

Vijaya Gopal
Italy
April 18-22, 2016

G Srinivas
USA
May 9-13, 2016

Anant Bahadur Patel
Singapore
May 7-13, 2016

Prof Amitabha Chattopadhyay
Greece
May 23-27, 2016

Mohammad Jafurulla
Greece
May 23-27, 2016

Prof Amitabha Chattopadhyay
Switzerland
June 12-17, 2016

G R Chandak
China
July 1-3, 2016

V Radha
Czech Republic
July 21-25, 2016

Jyotsna Dhawan
USA
July 24-29, 2016

Mandar V Deshmukh
Japan
August 21-26, 2016

R Sankaranarayanan
South Korea
September 4-8, 2016

Manjula Reddy
Czech Republic
September 14-17, 2016

K Thangaraj
Italy
September 16-19, 2016

K Thangaraj
Australia
October 11-16, 2016

Rakesh K Mishra
South Korea
October 12-14, 2016

Mandar V Deshmukh
Germany
September 4-8, 2017

Jyotsna Dhawan
France
September 19, 2017

Umapathy
Russia
March 19 - 31, 2017

Conferences, Symposia & Training Programmes

The Annual Meeting of "Society for Neurochemistry India (SNCI) & Workshop"

The National Conference on "Recent trends in Neurological and Psychiatric Research" was held from December 9 to 11, 2016, followed by a Workshop on "Modern Molecular Techniques to study Neurological and Psychiatric Disorders" from December 12 to 16, 2016. There were interesting lectures by 33 faculty from various prestigious research institutions and hospitals in the country, attended by 133 participants from various universities, colleges and research institutions in the country. There was a cultural program 'PANCHABHOOTAS' by Shri Sanjay Kumar Joshi and disciples December 10, 2016.

Conference on "Next Generation Sequencing, 22 - 24 Feb 2017"

The CSIR-CCMB and the Bioserve Biotechnologies (India) Private Limited, jointly organized the Second Conference on Next Generation Sequencing from February 22 to 24, 2017, in the CCMB. The conference involved lectures on Animal Genomics, Plant Genomics and Cancer Genomics. In the second half of the February 22, 2017, the CCMB Founder's Day was celebrated marking the 89th birthday of CCMB's founder Director, Dr. Pushpa Mitra Bhargava.



Workshop on "High-resolution Respirometry: February 07 - 08, 2017"

The CSIR-CCMB and the SMRM, OROBOROS Instruments (Austria), had jointly organized a workshop on High Resolution Respirometry for 20 participants from February 07-08, 2017, in the CCMB. The workshop involved a lecture by Dr. Robert Pitceathly, UCL, London on "Clinical and molecular characteristics of adult mitochondrial disorders" and Demo- experiment on High-resolution Respirometry, discussion on hands on data analysis and Q&A session on design of experimental protocols.

6th Meeting of Asian Forum for Chromatin and Chromosome Biology: March 03-05, 2017

Since 1996, the CSIR-CCMB and the Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bengaluru, have been alternately hosting the biennial meetings of the Asian Forum for Chromatin and Chromosome Biology. The 6th Meeting was hosted by the CSIR-CCMB from March 03 to 05, 2017 in the CCMB. 193 participants including 30 National and International Speakers and students, postdoctoral fellows from India and abroad participated the meeting, which involved 21 lectures and 16 short talks.

3-day hands on training workshop on DNA-based wildlife forensic diagnostics: 23-25 Mar 2017

CCMB-LaCONES is playing an important role in generating meaningful DNA evidence for law enforcement agencies to unambiguously identify species targeted in poaching and illegal wildlife trade since 2000. Partnership with the line agencies involved in controlling and preventing wildlife crimes is crucial for effective exchange of information. Based on this, a 3-day hands-on training/

workshop -“DNA-based wildlife forensic diagnostics”, was conducted at CCMB Annexe-I (LaCONES) from March 23 to 25, 2017. There were 11 participants from Wildlife Institute, colleges and government organizations, who got an opportunity of hands-on practical training on biological sample collection, preservation, DNA isolation, quantification, PCR amplification, DNA sequencing and data analysis for species identification, individual identification, relatedness and sexing.

Events & Popular talks

Launch of iHUB

The CSIR-CCMB launched its Innovation Hub [iHub] on October 07, 2016, to facilitate research and development intended for societal impact. The Hub is situated about a kilometer from the CCMB main campus, has about 40000 sft fully equipped laboratory space and a dedicated area for plant breeding. The launch of iHub was called as “Meet the Innovators” (MI), an event which is planned to take place at least three times a year. Dr. K I Varaprasad Reddy, Founder and CMD of Shantha Biotech, and the first ever incubator of CCMB, was invited as Chief Guest to the event.

On this occasion, MoUs were exchanged with the National Research Development Corporation [NRDC, New Delhi] and with M/s. Theranos Life Sciences Private Limited, Hyderabad; a letter of interest was signed with M/s. Bioserve Biotechnologies India Private Limited.

Later, a panel discussion on “Valley of Opportunity for Innovation and Technology Development” was conducted with Dr. S. Ramaswamy (InStem, Bengaluru), as the Moderator and Dr. Gayatri Saberwal (IBAB, Bengaluru), Dr. Taslimarif Saiyed (C-CAMP, Bengaluru), Dr. Rama Iyer (T-Hub, Hyderabad) and Dr. T. S. Sridhar (St. John’s Research Institute, Bengaluru), as the panelists.

Pre IISF Events (November 1-11, 2016)

As a part of India International Science Festival (IISF-2016), under the Ministry of Science and technology and Ministry of Earth sciences in association with Vijynana Bharati (VIBHA), several program from November 1 to 15, 2016, at the CSIR-CCMB. Invitations were sent to schools/colleges and

exclusive events were executed for students of different schools/colleges from in and around Hyderabad from 10 AM to 5 PM. The program included visit to laboratories, Exhibition of technologies developed at CCMB, products developed by CCMB and interaction with the faculty, research staff and students.

Foundation Day (November 26, 2016)



The present laboratory complex of the CSIR-CCMB was dedicated to the nation and to the cause of science on November 26, 1987 by the then Prime Minister Shri Rajiv Gandhi. Ever since, the tradition of celebrating the Foundation Day on the 26th of November is being carried out by inviting a Nobel Laureate or a Distinguished Scientist or an internationally renowned Scientific Expert, for delivering the Foundation Day lecture. The 29th Foundation Day lecture on November 26, 2016, was delivered by Dr. Girish Sahnii, Director-General, CSIR and Secretary, Department of Scientific and Industrial

Research (DSIR) on “Putting Science to Public Use: A Personal Perspective”.

The CSIR-CCMB Annual Report 2015-16 was released on this occasion. This was followed by the 'Foundation Day Symposium' of CCMB PhD students, a half day event where the Ph.D. students of the lab present a seminar on their research work. On this day, the CSIR-CCMB awards the Anindya Kumar Ghosh Memorial Award to the PhD students (Junior Research Fellows) who have secured the highest score in the previous year's Student Coursework Seminar. From the 2015 batch students, the award was presented to:

- Ms. Komal Ashok Awalellu
- Mr. Debabrata Jana

Networking Meeting (iHUB& IKP) (January 18, 2017)



The CCMB-iHUB signed an MoU with the ICICI Knowledge Park (IKP), as part of which, IKP shall hold mentoring sessions and conduct workshops for innovators at CCMB-iHUB, handhold stakeholders in the innovation ecosystem viz. IP, Technology, investors etc., nurture the innovators for building entrepreneurship and facilitate services of Analytical Facility at IKP to the incubates and associated innovators of CCMB-iHUB. In line with the MoU, a Networking meeting on “Funding in Life sciences & Healthcare: Innovation Grants for Entrepreneurship” was organized by the CCMB-iHUB and IKP in the Medical Biotechnology Complex (CCMB Annexe-II), Uppal Road, Hyderabad on January 18, 2017. Dr. Rakesh K Mishra, Director, CSIR-CCMB and Dr. Dipanwita Chattopadhyay, Chairman & CEO, ICICI Knowledge Park, addressed the young entrepreneurs on this occasion.

This was followed by exchange of MoUs with the CCMB-iHUB and with Kommareddi Biopharma Pvt Ltd. In the event, Dr. Srinivas Prakash Regalla, Professor, BITS-Pilani Hyderabad Campus also discussed a case study on his Entrepreneurial Journey in Academia.

DG ICMR visit (February 06, 2017)

Dr. Sowmya Swaminathan, Secretary to the Government of India (Department of Health Research), Ministry of Health & Family Welfare & Director General, Indian Council of Medical Research (ICMR), visited the CSIR-CCMB on February 06, 2017. The DG, ICMR met the faculty of the CSIR-CCMB followed by a visit to the Clinical Research Facility (CRF), CCMB Annexe-II.

Kurt Wuthrich Talk- PJC lecture (February 08, 2017)

As part of the Council of Scientific and Industrial Research (CSIR) platinum jubilee celebrations (2016-17), Nobel Laureates or distinguished world Scientists/ Academicians/ Technologists of that level were to be invited to visit different CSIR Laboratories and deliver lectures. The CSIR-CCMB invited Prof. Kurt Wüthrich, Nobel laureate in Chemistry, 2002, to deliver a talk at the CCMB as part of the CSIR@75 celebrations.



Prof. Wüthrich visited the CSIR-CCMB on February 08, 2017, and delivered a talk on “My Life as a Scientist: 50 years in Basic Research”. During his visit to the centralized facilities, Prof. Wüthrich was excited to note that the biomolecular NMR facility of CCMB is very well-maintained and expressed high level of satisfaction after learning some of the scientific contributions made by this facility.

Founder's Day (February 22, 2017)



The Centre for Cellular and Molecular Biology (CSIR-CCMB) was a result of vision, foresight and relentless efforts of its founder, Dr. Pushpa Mitra Bhargava (PMB), who wanted to put Indian science in the forefront of cellular and molecular biology. From the year 2017, to reminisce the relentless efforts of Dr. PMB, the CSIR-CCMB began celebrating February 22nd (birth date of Dr. PMB) as its Founder's Day. The first CSIR-CCMB Founder's Day lecture was delivered by Dr. T Ramasami, Former Secretary, Department of Science and Technology (DST), Government

of India, on 'Triple Helix of Excellence, Relevance and Sustenance'. The talk was followed by Aakruti Kathak Kendra's Taal Vinyas (Journey of Rhythm) composed by Shri Raghav Raj Bhatt.

International Women's day

In view of International Women's Day on 8th March 2017, CCMB Staff Club organized two events for Women Employee of CCMB (Staff, Students & their family members).

1. A Quiz Competition on 7th March 2017 :

A fun-based quiz with interesting facts mostly confined to 'Indian Women' was conducted covering all about their achievements and current affairs.

2. Two Talks on 8th March 2017 :

- A) A "Health Talk" on general health issues and awareness ranging through all age groups was given by Dr. P. Usha Rani (HOD for Obstetrics and Gynecology Department, Yashoda Hospital, Secunderabad), specialist in Laparoscopy, Infertility & High Risk Pregnancy with 33 yrs of experience & skilled in managing complex & critical patients. As HOD taught & trained scores of students who are presently working all over India.
- B) A short documentary film on "Women in today's World" was screened. Also a talk entitled "If a women has the will power, she can excel in any field from culinary to space" was delivered by Ms. Sandhya Janak (Science Journalist cum actress) who was a freelance Science journalist (the Indian Express) with 10 years experience in writing and covering Scientific research in Indian Science and its application in various fields. Later she became a successful Telugu film character artist and acted in more than 150 Telugu films.



World Down Syndrome Day (March 21, 2017)

On March 21, 2017, World Down Syndrome Day was observed at the Centre for Cellular and Molecular Biology [CSIR-CCMB]; jointly organized by Asian Institute of Fetal Medicine & Reproductive Genetics [AIFMRG], Hyderabad. Meeting was covered by scientific session and comprised

presentations with significant contributions by eminent doctors working in the areas of Down Syndrome- Diagnosis, Care and Management. The uniqueness of this meeting is that the scientific session overlapped with Paint/Sketch hour for the children attended by parents and teachers from Institute of Genetics & Hospital of Genetic Diseases.



Staff, Research Students, Project Staff & Others as on November 01, 2017

SCIENTIFIC GROUPS

GROUP LEADERS

Anant B Patel Group

Anant B Patel	Principal Scientist
K S Vardarajan	Technical Assistant
Pravin Kumar Mishra	Ph.D. extension
Narayan Datt Soni	SRF
Dipak Roy	JRF
Salil Saurav Pathak	Project SRF
Kamal Saba	Project Assistant-III
Madhuri Puvvada	Project Assistant-II
Sreemantula Arun Kumar	Project Assistant-II
Shoumik Roy	Project Assistant-II
Pooja Gautam	Project JRF
Sampath Kumar T K	Project JRF

Arvind Kumar Group

Arvind Kumar	Senior Scientist
Sachin Singh	Scientist
Avijeet Kamle	Project Investigator
Shams Ul Haq Talee	SRF
Unis Ahmad Bhatt	SRF
Nitin Khandelwal	Research Associate-I

G R Chandak Group

G R Chandak	Sr Principal Scientist
K Radha Mani	Principal Tech Officer
Seema Bhaskar	Senior Tech Officer (3)
Inder Deo Mali	Lab Assistant
P Ashok	Lab Attendant (2)
Meraj Ahmad	Ph.D. extension
N Suraj Singh	Ph.D. extension
Dilip Kumar Yadav	Ph.D. extension
Prachand Issarapu	SRF
Ashutosh Singh Tomar	SRF
Lovejeet Kaur	Research Associate-I
Ajay Deepak Verma	Research Associate-I
Radhika P Ramachandran	Project Assistant-III
Vishlavath Jyothi	Project Assistant-II
Ramya B	Project Assistant-II
Swathi Bayyana	Project Assistant-II
Pujitha Kommineni	Project Assistant-II
Vinay Donipadi	Project Assistant-II
Challapalli Mounika	Project JRF
Sara Sajjadi	Project JRF
Akshay Dedaniya	Project JRF

Jyotsna Dhawan Group

Jyotsna Dhawan	Chief Scientist
Nandini Rangaraj	Sr Principal Scientist
Ch Sudhakar	Sr Principal Scientist
C Subbalakshmi	Principal Tech Officer
Suman Bhandari	Technical Asst [FACS]
Sujoy Deb	SRF
Debarya Saha	SRF
Swetha S	JRF
Priti A S	JRF
Gunasekaran Subramaniam	Research Associate-I
Gunjan Purohit	Research Associate-I
Prabhavathy Devan	Research Associate-I
Lamuk Zaveri	Project Assistant-III
Amena Saleh	Project Assistant-III
Ananga Ghosh	Project Assistant-II
Ajoy Aloysius	Project SRF
Swetha Gopal	Project JRF

K Thangaraj Group

K Thangaraj	Sr Principal Scientist
Nitin C Tupperwar*	Senior Scientist
G Mala	Senior Tech Officer (3)
S Deepa Selvi Rani	Senior Tech Officer (2)
Jagamohan Chhatai	Technical Assistant
D V S Sudhakar	Ph.D. extension
Sunil Kumar Tripathi	SRF
Rajan Kumar Jha	SRF
Nipa Basak	SRF
Jaydeep A Badarukhiya	SRF
Lomous Kumar	SRF
Umesh Kumar	JRF
Deepak Kumar Kashyap	JRF
Paramasivam A	Research Associate-I
Narmada	Research Associate-I
[* on EOL]	

Karthikeyan Vasudevan Group

Karthikeyan Vasudevan	Senior Principal Scientist
B Sambasiva Rao	Senior Scientist
P Anuradha Reddy	Scientist
Y V Subba Lakshmi (Canteen)	Sr Tech Officer (1)
S Harika	Technical Assistant

K Rajya Lakshmi	Technical Assistant
Afsar Sogra	Lab Assistant
Snehalatha Vadigi	DST Inspire Faculty
Ashish Jha	SRF
Siddharth Bhatia	SRF
Gayathri Sreedharan	JRF
Ravi Kumar Singh	JRF

Manjula Reddy Group

Manjula Reddy	Sr Principal Scientist
G S N Reddy	Principal Tech Officer
S Venugopal	Senior Technician (2)
T Apuratha Pandiyan	Ph.D. extension
Nilanjan Som	SRF
Pavan Kumar Ch	SRF
Raj Bahadur	JRF
Shambhavi Garde	JRF
Anju Bala	Project RA
Balaji V	Project RA
Moneca Kaul	Project Assistant-II

Puran Singh Sijwali Group

Puran Singh Sijwali	Senior Scientist
S Thanumalayan	Senior Tech Officer (2)
Nandita T	SRF
Renu Sudhakar	SRF
Manish Bhattacharjee	SRF
Deepak Kumar	JRF
Zeba Rizvi	JRF
Neeradi Dinesh	Research Associate-I
Amit Kumar Nagwani	Project JRF
Divya Das	Project JRF

Rajan Sankaranarayanan Group

Rajan Sankaranarayanan	Chief Scientist
P Shobha Krupa Rani	Senior Scientist
Biswajit Pal	Senior Scientist
R Rukmini	Senior Tech Officer
P Sambhavi	Technical Assistant
K Malleshm	Technical Assistant
Patil Gananan Shrikant	SRF
Santosh Kumar K	SRF
Mazeed Mohammad	SRF
Rajkanwar Nathawat*	SRF
Satya Brata Routh	SRF
Jotin Gogoi	JRF
Sudipta Mondal	JRF
Salam Herojeet Singh#	Research Associate-I
Katta Suma	Research Associate-I

Raghvendra Singh	Research Associate-I
K Priyadarshan	Research Associate-I
Murali Krishna Madduri	Project JRF
Bhavita Kattula	Project JRF
Priyanka Dahate	Project JRF
Ayshwarya S	Project JRF
Subhash Narasimhan	Project JRF

[*Joint student with Dr. Ramesh V Sonti]

[#Joint student with Dr. Rakesh K Mishra]

Rakesh Kumar Mishra Group

Rakesh Kumar Mishra	Director
Rashmi Upadhyay Pathak	Principal Scientist
A Srinivasan	Senior Tech Officer (1)
Runa Hamid	DST-Project Investigator
Divya Tej Sowpati	Project Scientist
Surabhi Srivastava	Project Scientist
Gopal Kushawah	Ph.D. extension
Titus Surendra Ponrathna	Ph.D. extension
Avinash Srivastava	SRF
Shagufta Khan	SRF
Phanindhar K	SRF
Parna Saha#	SRF
Rahul Sureka	SRF
Nikhil Hajirnis	JRF
Ashish Bihani	JRF
Ravina Saini	JRF
Mohammed Arif	Research Associate-III
Mukesh Pratap Yadav	Research Associate-I
Shreekant Verma	Research Associate-I
Salam Herojeet Singh*	Research Associate-I
Chakrapani Vemulawada	Research Associate-I
Aishymaa Yusef M M Hassan	TWAS PG Fellow
Ishanee Srivastava	Project Assistant-II
Avvaru Akshay Kumar	Project Assistant-II
Nisha M	Project Assistant-I

[*Joint student with Dr. R Sankaranarayanan]

[#Joint student with Dr. Shrish Tiwari]

Ramesh K Aggarwal Group

Ramesh K Aggarwal	Chief Scientist
V Purushotham	Technical Assistant
Ch Viswanatham	Lab Assistant
Chhavi Dhawar	JRF

Ramesh V Sonti Group

Ramesh V Sonti	Chief Scientist
Hitendra Kumar Patel	Senior Scientist

P Ramesh	Principal Tech Officer
Raju Madanala	Senior Tech Officer (1)
M B Madhavi	Tech Officer
B Kranthi	Technical Assistant
K Bipin Kumar	Ph.D. extension
Vishnu Narayanan M	SRF
Kamal Kumar Malukani	SRF
Shakuntala E Pillai	SRF
Neha Rajendra Kachewar	SRF
Sohini Deb	SRF
Komal Ashok Awalellu*	JRF
Gokulan C G	JRF
Roshan M V	Project RA
Hota Shiva Jyothi	Project Assistant-II
Shailaja Kanumuri	Project Assistant-II
Sridivya V	Project Assistant-II

(*Joint student with Dr. Imran Siddiqi)

Satish Kumar Group

Satish Kumar	Chief Scientist
Alok Kumar	Ph.D. extension
Rajesh V Iyer	SRF
Manikandan Mayakannan	Research Associate-I
Sivapriya Pavuluri	Research Associate-I
Sadiya Parveen	Project SRF
Bhim Bahadur Biswa	Project JRF

Tushar Vaidya Group

Tushar Vaidya	Sr Principal Scientist
Loka Ram Prasad	SRF
Pradyumna Swanand	
Paranjape	SRF
Devi Prasad V	SRF
Salunkhe Satyajeet Sunil	SRF

V Radha Group

V Radha	Chief Scientist
Bh Muralikrishna	Principal Tech Officer
M Sanjeev Chavan Nayak	Technical Assistant
G Vidyasagar	Lab Attendant (1)
Dhruv Kumar	Ph.D. extension
Divya S	SRF
Ramulu Ch	Research Associate-I
Kunal Dayma	Research Associate-I

Yogendra Sharma Group

Yogendra Sharma	Chief Scientist
R Phanindranath	Senior Tech Officer (1)

Syed Sayeed Abdul	Lab Attendant (1)
Anand Kumar Sharma	SRF
Asmita Dhansing Pawar	SRF
Radhika Khandelwal	SRF
Aditya Anand Jamkhindikar*	SRF
Amrutha H C	JRF
Venu Sankeshi	Research Associate-I
Shanti Swaroop Srivastava	Research Associate-I
Sai Uday Kiran P	Project Assistant-III

[*Joint student with Dr. R Sankaranarayanan]

PROJECT LEADERS

A S Sreedhar Group

A S Sreedhar	Principal Scientist
A Vijaya Lakshmi	Principal Scientist
K R Paithankar	Principal Tech Officer
Akhil Kotwal	Ph.D. extension
K Abhijnya Vijayavittal	Ph.D. extension
Pankaj Kumar	SRF
Satish Siripini	Project Assistant-II
Guntipally Mounika	Project JRF

Ajay Gaur Group

Ajay Gaur	Principal Scientist
A Sreenivas	Tech Officer
Siuli Mitra	Research Associate-I
Vaishnavi K	Project Assistant-III
Parmar Drashti Rameshbhai	Project Assistant-II
Venkata Charan Kumar B	Project Assistant-II

Amit Asthana Group

Amit Asthana	Principal Scientist
Ira Bhatnagar	Senior Scientist
Shahila Parween	Research Associate-I
Sourabh Kumar Srivastava	Research Associate-I
Lavleen Bhati	Project Trainee

G Umapathy Group

G Umapathy	Principal Scientist
Vinod Kumar	Technical Assistant
M S Ram	Ph.D. extension
Mihir Trivedi	JRF
Deepanwita Purohit	Project SRF
Gopi Suresh Oggu	Project Assistant-III
Raja Beaulah Budithi Neema	Project Assistant-III
Mahendar Reddy D	Project Assistant-II
Abirami K	Project Assistant-I

K Guruprasad Group

K Guruprasad	Sr Principal Scientist
A Sharada Devi	Principal Tech Officer
Pallabini Dash	Project Assistant-II

K Lakshmi Rao Group

K Lakshmi Rao	Principal Scientist
M K Kanakavalli	Senior Tech Officer (1)
O V Padmalatha	Senior Tech Officer (1)

H H Krishnan Group

H H Krishnan	Senior Scientist
M Mohan Singh	Technical Assistant
Manish Kumar Johri	Ph.D. extension
Sana Parveen	SRF
Dhiviya V	SRF
Haripriya Parthasarathy	JRF
Divya Gupta	JRF
Hitha G Nair	Project Assistant

Lekha Dinesh Kumar Group

Lekha Dinesh Kumar	Principal Scientist
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M Mohammed Idris Group

M Mohammed Idris	Principal Scientist
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M V Jaganadham Group

M V Jaganadham	Sr Principal Scientist
Deepika	Ph.D. extension

Mandar V Deshmukh Group

Mandar V Deshmukh	Senior Scientist
Upasana Rai	SRF
Sneha Paturi	JRF
Aute Ramdas Annasaheb	Project Assistant-II

P Chandra Shekar Group

P Chandra Shekar	Senior Scientist
G Srinivas	Senior Tech Officer (1)
Kale Hanuman Tulashiram	SRF
Debabrata Jana	JRF
Vishnu Vijay	JRF
Usha Kabilan	Project Assistant-II
Malini S	Project JRF

Palani Murugan Rangasamy Group

Palani Murugan Rangasamy	Senior Scientist
Ashis Kumar Pradhan	SRF
Babu R	Project Assistant-II

Raghunand R Tirumalai Group

Raghunand R Tirumalai	Senior Scientist
Amit Kumar*	Technical Assistant
Vimal Kishore	Ph.D. extension
Ravi Prasad Mukku	SRF
Mamata Sumant	
Savanagouder	Project Assistant-II
[* BSL II/III facility]	

Sadanand D Sontakke Group

Sadanand D Sontakke	Senior Scientist
Sujata Dev	Project Assistant-II

Shashi Singh Group

Shashi Singh	Chief Scientist
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Shrish Tiwari Group

Shrish Tiwari	Principal Scientist
Parna Saha*	SRF
Deepti Rao	JRF
Shubhankar Dutta	Project Assistant-II
[*Joint student with Dr. Rakesh K Mishra]	

Sunil Kumar Verma Group

Sunil Kumar Verma	Principal Scientist
Sanjay Kumar Suman	Technical Assistant

SCIENTIST FELLOWS

Venkata R Aditya Chalamcharla Group

Venkata R Aditya Chalamcharla	Project Scientist
Anubhav Bhardwaj	Technical Assistant

Mukesh Lodha Group

Mukesh Lodha	DBT Ramalingaswami Fellow
Akanksha Garhewal	SRF
Preethi Jampala	JRF
Aditya Undru	JRF
Supriya Sarma	Research Associate-I
Asha Ganpatrao Mane	Project JRF

Swasti Raychaudhuri Group

Swasti Raychaudhuri	DBT Ramalingaswami Fellow
Shivali Rawat	SRF
Shemin Mansuri	JRF
Bodhisattwa Saha	Research Associate-I
Debodyuti Mondal	Project Assistant-II

Valpadashi Anusha Project Assistant-II
Swathi Chadalwada Project JRF

Kumaraswamy Regalla Group

Kumaraswamy Regalla Wellcome Trust
DBT Fellow
Abishek Bharadwaj JRF
Manisha Kumari Sahu Project JRF

J C BOSE FELLOWS

Amitabha Chattopadhyay J C Bose Fellow
Sandeep Shrivastava* Senior Tech Officer (1)
Mohammad Jafurulla* Senior Tech Officer (1)
Parijat Sarkar SRF
Aditya Kumar G SRF
Bhagyashree D Rao SRF
Sreetama Pal SRF
Sukanya Bhowmick Project Assistant-II
Nikita Prakash Chutake Office Assistant
[*Reporting Dr.V Radha for Administrative purposes]

Ch Mohan Rao J C Bose Fellow
Kranthi Kiran Akula Ph.D. extension
Budnar Prashanth Ph.D. extension
Kamakshi Dandu SRF

Ghanshyam Swarup J C Bose Fellow
Akhouri Kishore Raghawan Ph.D. extension
Shivranjani C Moharir SRF
Gopalakrishna R SRF
Sayyad Zuber W Quamaruddin SRF

Imran Siddiqi Group

Imran Siddiqi Chief Scientist
V Sreenivasulu Principal Scientist
V Subbaiah Principal Tech Officer
Davda Jayesh K Narsibhai Ph.D. extension
Aswan Nalli Ph.D. extension
A Venkata Pardha Sardhi Ph.D. extension
Saurabh Pandey Ph.D. extension
Frank Keith Max SRF
Survi Mahesh SRF
Aparna Singh Research Associate-III
Madhumita Dash Research Associate-I
Saddala Surendra Project Assistant-II
Vaitla Vinya Project Assistant-II
Kaladhar Bethoju Project Assistant-II

R Nagaraj J C Bose Fellow
Pratapa Gayatri Project Assistant-II

Veena Parnaik J C Bose Fellow
Richa Khanna Ph.D. extension
Vidhya Krishnamoorthy Ph.D. extension

EMERITUS SCIENTIST

Purnima Bhargava Emeritus Scientist
Geethanjali Ravindran Research Associate I
Ashutosh Shukla Ph.D. extension

CONSULTANT SCIENTIST

N Madhusudhana Rao Consultant Scientist
Sankara Rao Kola Ph.D. extension
Tushar Ranjan Moharana Ph.D. extension

Director Group

Suman Siddharth Thakur Senior Scientist
Prachi Singh Scientist

iHUB

Jyotsna Dhawan In-charge
Archana Bharadwaj Siva Principal Scientist
K Rajkumari (innovation cell) Sr Principal Scientist
N Nagesh (innovation cell) Sr Principal Scientist
P Kavin Kennedy Sr Principal Scientist
Jomini Liza Stephen Senior Scientist
Manoj Balyan Senior Scientist
C B Tripura Sundari Scientist
Challa Venkatapathi Project Assistant-II
B Kiran Kumar Scientist
V Srinivas Principal Tech Officer
V Anuradha Senior Technician
Hemalatha Senior Steno
K Srinath Lab Attendant (1)
Dayalu D Job Contract

ON LIEN/ DEPUTATION

Sandeep Goel Principal Scientist
Shailendra K Saxena Senior Scientist
Marshal Dayal Scientist

ACADEMICS

Academic Cell
Manjula Reddy Coordinator
Puran Singh Sijwali Coordinator
V Anitha Project Assistant-II

Ph.D. students on Lab Rotation

Bedaballi Dey
Harshit Vaish
Pradeep Kumar
Jaydeep Paul
Harsh Joshi
Sagnik Dhar
Sivakumar P
Shraddha Vijay Lahoti
Annapoorna P Karthyayani
Mansi Srivastava
Priyanka Pant

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 Attendant (2)
R Siddaramappa	Lab Attendant (2)
K Raju	Lab Attendant (2)
B Lalaiah	Lab Attendant (1)
M Rajeshwari	Multitask Staff

Drosophila Facility

Rashmi Upadhyay Pathak	Principal Scientist
V Bharathi	Senior Tech Officer (1)
K Ramachandra Rao	Tech Officer
P Sabitha	Lab Attendant (1)

Proteomics Facility

Swasti Raychaudhuri	DBT Ramalingaswamy Fellow
V Krishna Kumari	Principal Tech Officer
C Sivakama Sundari	Principal Tech Officer
B Raman	Senior Tech Officer (3)
Y Kameshwari	Senior Tech Officer (3)

Tissue Culture Facility

V Radha	Chief Scientist
Ch Varalakshmi	Principal Tech Officer
V R Sundereswaran	Principal Tech Officer
Zareena Begum	Principal Tech Officer
B V V Pardhasaradhi	Senior Tech Officer (3)
T Avinash Raj*	Technical Officer
S Easra	Senior Technician (2)
P Venugopal	Lab Attendant (2)
T Dayakar	Lab Attendant (1)

[*Histology lab]

Transgenic Knockout Facility

P Chandra Shekar	Senior Scientist
B Jyothi Lakshmi	Senior Tech Officer (1)
D Partha Sarathi	Senior Tech Officer (1)
S Purnima Sailasree	Tech Officer
Asha Kumari	Technical Assistant

Zebrafish Facility

Rashmi Upadhyay Pathak	Principal Scientist
K Ravinder	Principal Tech Officer
M L Arvinda Swamy	Technical Assistant
P Shivaji	Senior Technician (2)
K Sashi Kumar	Lab Assistant
Damera Santhosh Kumar	Project Assistant-I

Support Facilities

Planning, Monitoring and Evaluation (PME)	
M R Vishnu Priya	Sr Principal Scientist
B V Ramakrishna	Senior Tech Officer (3)
M V Subba Rao	Junior Steno
K Satyanarayana	Lab Assistant
Gulzar Khan	Lab Attendant (1)
Pulipati Sadguru	Project Assistant-II

Human Resources Group (HRG)

Archana B Siva	Principal Scientist
R Leela Kumari	Principal Tech Officer

Business Development Group (BDG)

K Lakshmi Rao	Principal Scientist
P S Raju	Principal Tech Officer
K Anitha	Technician (1)

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
P Vanaja	Principal Tech Officer
J D Fernandes	Senior Tech Officer (3)
Mahesh Prasad	Senior Tech Officer (3)
U S T R B Bapi Raju	Senior Tech Officer (3)
B Venkata Narayana	Senior Tech Officer (2)
N Ravindra Chakravarthi	Senior Tech Officer (1)
Sudatt T Tambe	Senior Tech Officer (1)
Dattatrya N Gurkhel	Senior Tech Officer (1)
A Harikrishna	Senior Tech Officer (1)
K Sanjeev Kumar	Senior Tech Officer (1)
A Bala Murugan	Senior Tech Officer (1)
A Syam Kumar	Senior Tech Officer (1)
A Narsing Rao	Private Secretary
Devender Sundi	Technical Assistant
Arpita Maji	Technical Assistant
Chetan R Khapekar	Technical Assistant
D Ramesh	Senior Technician (2)
YSSV Prasad	Job Contract

Information Technology (IT) Group

Geetha Thanu	Senior Scientist
Sublari Balaraju	Scientist
Aparna Kumari	Scientist
Biswajit Roy	Scientist
P Nagalinga Chary	Senior Tech Officer (3)
P Radhakrishna Murthy	Senior Tech Officer (3)
K Sambasiva Rao	Senior Tech Officer (2)
N Siva Rama Prasad	Senior Tech Officer (2)
A Padmavathi	Senior Tech Officer (1)
S Mahalingam	Senior Tech Officer (1)
K Rama Chary	Senior Tech Officer (1)
Y Padmavathi	Senior Steno
M Srinivasa Rao	Lab Attendant (1)
Sreekanth Mamidala	Project Assistant-II

Laboratory Technical Services

Y V Rama Rao	Senior Suptd Engineer
G Rajendra Prasad	Suptd Engineer
Mani Ramana Rao	Senior Steno
S Krishna	Lab Assistant
P Gyaneshwar	Lab Assistant
L Laxman Dora	Lab Assistant
M A Jaleel	Lab Attendant (2)
B Sanjeeva Rao	Lab Attendant (2)

Library & Documentation

V V Hanumaprasad	Principal Tech Officer
P Nageshwara Rao	Senior Tech Officer (3)

Science Communications & PR

V Vijaya Bhaskar	Principal Scientist
B V Ramakrishna	Senior Tech Officer (3)

Horticulture

A Veerabhadra Rao	Chief Scientist
K Gopichand	Senior Technician (2)
M M Rajendran	Lab Assistant

Head-Engineering Services

G C Thanu	Senior Suptd Engineer
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AC, Refrig & Elec Engineering

B Vijaya Kumar	Asst Exe Engineer
K Nagendrababu	Asst Exe Engineer
A Varaprasada Rao	Asst Exe Engineer
V Prabhakar	Senior Technician (2)
N Rajan	Senior Technician (2)
K Prabhakara Rao	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)
V Prabhakar	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)
S Venkata Sastry	Lab Assistant
P Venkatarama Rao	Lab Assistant
V Chandramohan	Lab Assistant
A V Ramakrishna Reddy	Lab Assistant
T Venkateswar Rao	Lab Assistant
M Mazhar Ali	Lab Assistant
K Sreeram	Lab Assistant
M Padmanabhan	Lab Assistant
K Nagabhushanam	Lab Assistant
B M Nagesh	Lab Assistant
T Sambasiva Rao	Lab Attendant (1)

Civil Engineering

Ch Bikshamaiah	Suptd Engineer
Sheelwantayya	Suptd Engineer
Devidas M Nikhar Engineer	Assistant Exe
G Ramesh	Assistant (G) Gr.I
A Prem Kumar	Senior Technician (2)
M Tirumala Rao	Senior Technician (1)
Ananda S Paturkar	Senior Technician (1)
M Anjaneyulu	Lab Assistant
K Narayana	Lab Assistant

General Engineering

Ashok Baswa	Suptd Engineer
L Murali	Senior Technician (2)
R Koteswara Rao	Senior Technician (2)
B Venugopal	Senior Technician (2)
N Sudhakar	Senior Technician (2)
Suresh Prasad	Senior Technician (2)
K Venkatramana	Senior Technician (2)
N Nagabhushanam	Lab Assistant
B Balakrishna Reddy	Lab Assistant
N Narasinga Rao	Lab Assistant
K Y Das	Lab Assistant
Syed Khundmier	Lab Assistant
C Rosaiah	Lab Attendant (2)
V Shankar Rao	Lab Attendant (2)
B Satyanarayana	Lab Attendant (2)
P Srinivas	Lab Attendant (1)

ADMINISTRATION & MANAGEMENT

Director's Office

S Madhuri	Senior Tech Officer (2)
D Lavanya	Senior Tech Officer (1)
R V Subba Rao	Private Secretary
B V N Naveen Kumar	Technical Assistant
G Satyanarayana	Lab Attendant (2)

Administration

J Shankar Rao	Controller of Admin
P Sudha Rani	Administrative Officer
R Chandrasekhar	Hindi Officer
J Venu	Senior Tech Officer (1)
Sunil Kumar	SO (G)
Govind Kumar Jha	SO (G)
M Ranga Rao	Senior Steno
K Pratap	Assistant (G) Gr.II
Assunta Pradeep	Assistant (G) Gr.I
Shaheen Bano	Assistant (G) Gr.I

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 Surekha Devi	Assistant (G) Gr.I
T Rajani	Assistant (G) Gr.I
Ch Sridevi	Assistant (G) Gr.I
K Madhavi	Receptionist
Mahendra	Senior Technician (2)
B Srinivas	Senior Technician (2)
K Satyanarayana	Senior Technician (2)
K Venkateswar	Senior Technician (2)
G Anand	Senior Technician (2)
N Ravinder	Senior Technician (2)
Mohd Pasha	Senior Technician (2)
Mohd Ashrafuddin	Senior Technician (2)
V Malleshwar	Senior Technician (2)
R Nagabhushanam	Senior Technician (1)
Mahender Vynala	Technician (1)
B Madhusudhana Reddy	Lab Assistant
Mohd Gazanfar Ali	Lab Assistant
K Krishnamacharyulu	Lab Assistant
Ravindranath	Lab Assistant
B Venkateswarulu	Lab Assistant
Ch Chandrashekar	Lab Attendant (2)
J Anjaiah	Lab Attendant (2)
M Sharadha	Bearer (Adm)
C V S Padmaja	Bearer
Ambe Naveen Kumar	Multitask Staff
Ch Swapna	Job Contract

Finance & Accounts

A Prabhakar Rao	Fin. & Accounts Officer
Ch Vijaya	SO (F&A)
Vimala Prakash	Assistant (F&A) Gr.II
K Sujatha	Assistant (F&A) Gr.II
V V L Prasanna	Assistant (G) Gr.I
K Rama Krishna	Assistant (F&A) Gr.I
K Ganga Bhavani	Senior Technician (2)
W Sudhakar	Senior Technician (2)
M Vishnu Yadav	Technician (1)
K Venkateswarulu	Lab Attendant (2)
Nagamani A K	Project Assistant-I
Chinthala Praneeth Kumar	Data Entry Operator
Ramakrishna U	Job Contract
Swarajya Lakshmi G	Job Contract
S Anuradha	Job Contract

Stores & Purchase

Dharmendra Kumar	Stores & Purchase Off.
B Rajender Kumar	SO (S&P)
S S Lakshmi	Assistant (S&P) Gr.III
D V Ramana Murthy	Assistant (S&P) Gr.I
S Aruna	Assistant (S&P) Gr.I
D Balaji Prasad	Senior Technician (2)
K Sekhar Babu	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)
Malles R	Job Contract

Medical Services

G Sujatha	Medical Officer
A Mahesh	Technical Assistant
T Nagalakshmi	Technical Assistant
U V Sitaramamma	Senior Technician (2)
M R Ravindra Nath	Senior Technician (2)
R Palnitkar	Consultant
Ravinder Reddy D	Project Fellow
Nusrath Banu	Job Contract
Thirupathaiah G	Job Contract

Security

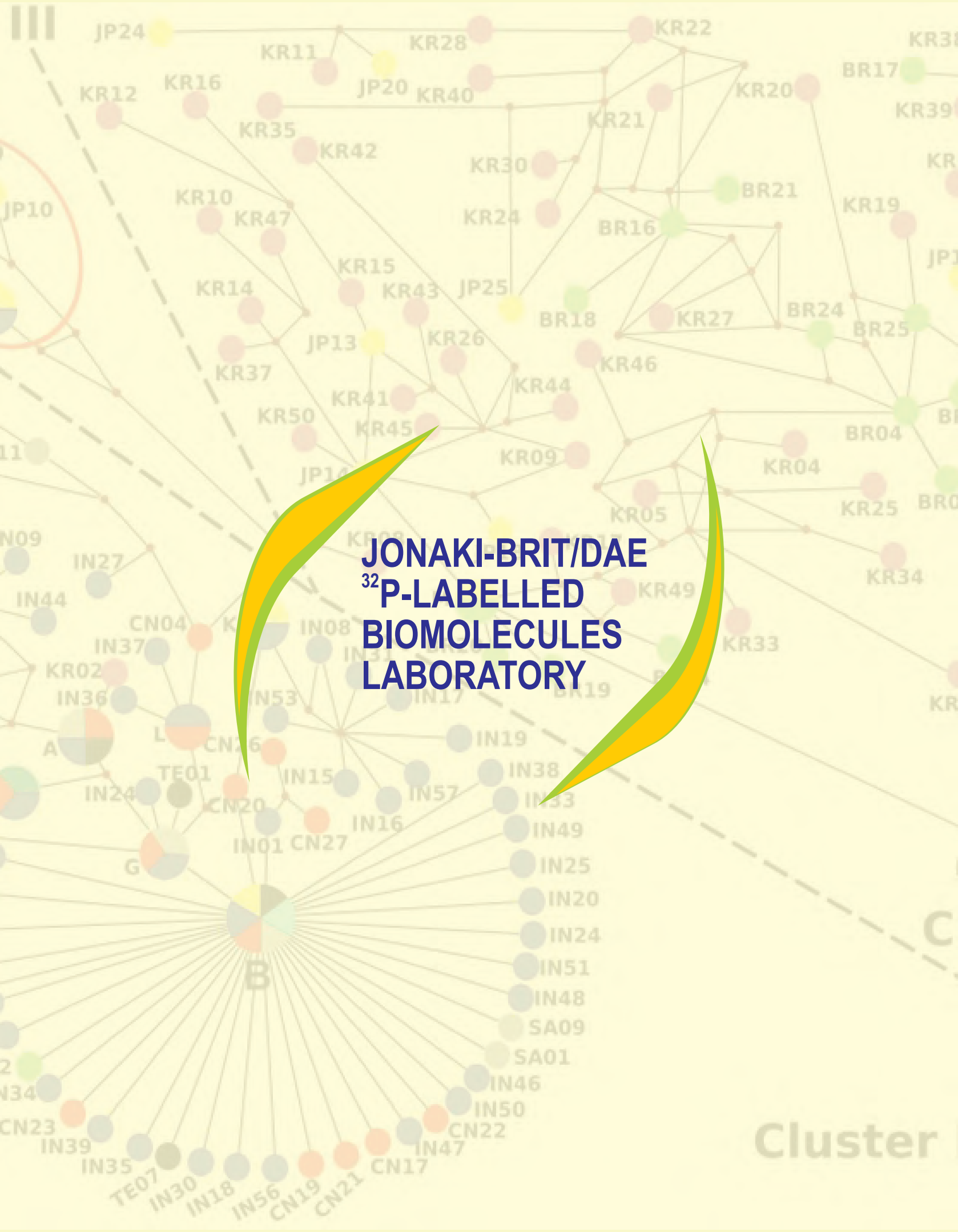
C V Tirumala Rao Security	Senior Security Officer
K V V S Raveendra Kumar	Security Officer

Guest House

Anil Kumar Sahu	Principal Tech Officer
G Christy Wilson	Senior Technician (2)
B Sadanandam	Senior Technician (1)
A Selvam	Senior Technician (1)
Mohd Jaffer	Lab Assistant
K Yadagiri	Lab Assistant
Raymond Peter	Job Contract

Canteen Group

Vikram Kumar	Technical Assistant
M Venkatesan	Senior Technician (2)
P M Mani Maran	Senior Technician (2)
Benedict	Senior Technician (2)
D Ramesh	Senior Technician (2)
K Ramesh Babu	Senior Technician (1)
M Devendra Nath	Senior Technician (1)
B M Narasing Rao	Lab Assistant
D Laxmi Devi	Lab Assistant
Mohd Athar Ali	Lab Attendant (2)
N Aruna	Lab Attendant (2)
M Tataji	Lab Attendant (2)
R Suresh Kumar	Lab Attendant (2)
Savitri Luhura	Lab Attendant (1)
S Yadaiah	Lab Attendant (1)
K Lakshmi	Bearer (Adm)



JONAKI-BRIT/DAE
³²P-LABELLED
BIOMOLECULES
LABORATORY

Cluster

VI. Regional Centre, Jonaki, BRIT

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 has been serving 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 $^{99\text{m}}\text{Tc}$ -radiopharmaceutical cold kits produced at Radiopharmaceutical laboratory of BRIT in Mumbai. Cold kits are for use in conjunction with $^{99\text{m}}\text{Tc}$ - Pertechnatate, in imaging of human organs for diagnosis and treatment, at the nuclear medicine centres of the hospitals and diagnostic centres in Telangana and Andhra Pradesh.

In order to expand the service, we will soon begin supply of $^{99\text{m}}\text{TcO}_4^-$ (sodium pertechnetate) from RC, BRIT, JONAKI. We are in the process of setting up laboratory for Radiopharmaceutical and Bio QC of $^{99\text{m}}\text{TcO}_4^-$ & its formulations. Further, we are in the final stages of obtaining license from AERB to start the facility.

We supply Taq DNA polymerase, PCR master mix, and Nucleic acid Isolation kits across the country on a regular basis.

Real time PCR Applications for Molecular Diagnostics is one of the research areas of the Regional Centre.

LIST OF PRODUCTS

1. ^{32}P Nucleotides:

CODE PRODUCT

101	^{32}P ATP
102	^{32}P dCTP
103	^{32}P dATP
104	^{32}P dGTP
106	^{32}P ATP
107	^{32}P GTP

RADIOACTIVE BIOCHEMICALS

108	^{32}P UTP
109	^{32}P CTP
1010	$[3',5'\text{-}^{32}\text{P}]p\text{CP}$
1011	^{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 6	^{35}S Glutathione
LCS 7	^{35}S Methionine-Cysteine mix Eleg mix
LCS 8	Protein <i>in vivo</i> twin label mix

3. Non-radioactive Biochemicals

CODE PRODUCT

LCK-1	Nick Translation Kit (20 reactions)
LCK-2	Random Primer Labelling Kit (30 reactions)
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)
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)
LCE-101	Taq DNA Polymerase Enzyme (100 Units)
LCE-102	Taq DNA Polymerase Enzyme (250 & 500 Units)
LCE-103	Taq DNA Polymerase Enzyme (1000-5000 Units)
LCE 104	Taq DNA Polymerase Enzyme (5001-50000 Units)

LCE 105	Taq DNA Polymerase Enzyme (50001 up to 99999 Units)
LCE 1000	Bulk packs more than 100000 units on enquiry
PMX 01	PCR Master Mix [100 Rxn (2 x 50)]
PMX 02PCR	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 bulk quantity (please enquire)

4. Cold kits for formulation of ^{99m}Tc-radiopharmaceuticals

Code	Kit for preparation of Tc-labelled Short form	Radio-pharmaceutical
TCK-5	Sulphur Colloid	^{99m} Tc S/C
TCK-7	Diethylene TriaminoPenta Acetic Acid	^{99m} Tc-DTPA
TCK-15	Glucosheptonate	^{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

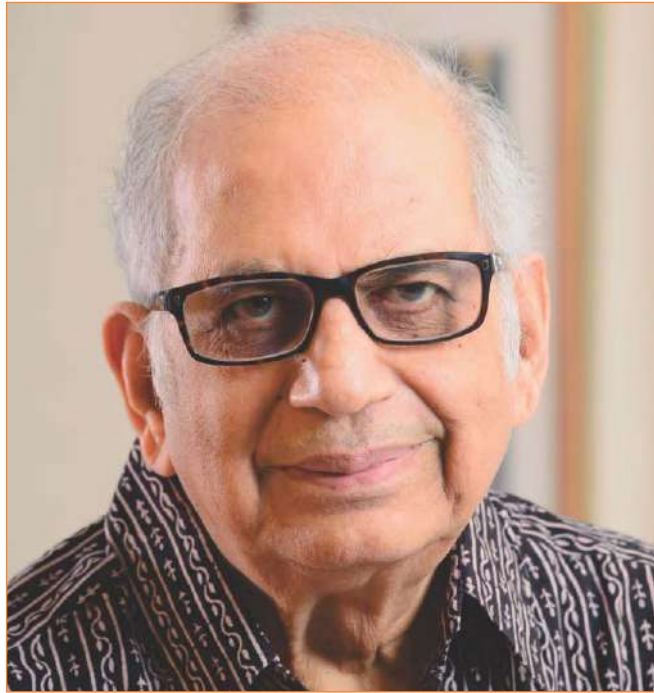
Staff of BRIT, JONAKI (as on 10-11-2017)

1. Smt Papia Hazra, Oic, Rc, Brit, Hyderabad
2. Dr. B.R. Varma, Manager
3. Dr. T.K. Sankaranarayanan, Manager
4. Shri M. Sreenivasulu
5. Shri N. Ambedkar
6. Smt. T. Raja Rajeswari
7. Shri S. Srikanth
8. Shri T.K. Sudhir
9. Shri M.B. Kumbhar
10. Shri P.B. Morey
11. Shri Jagdish Chandra
12. Shri S. Venkatesh
13. Shaik 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





Pushpa Mitra Bhargava (1928–2017)

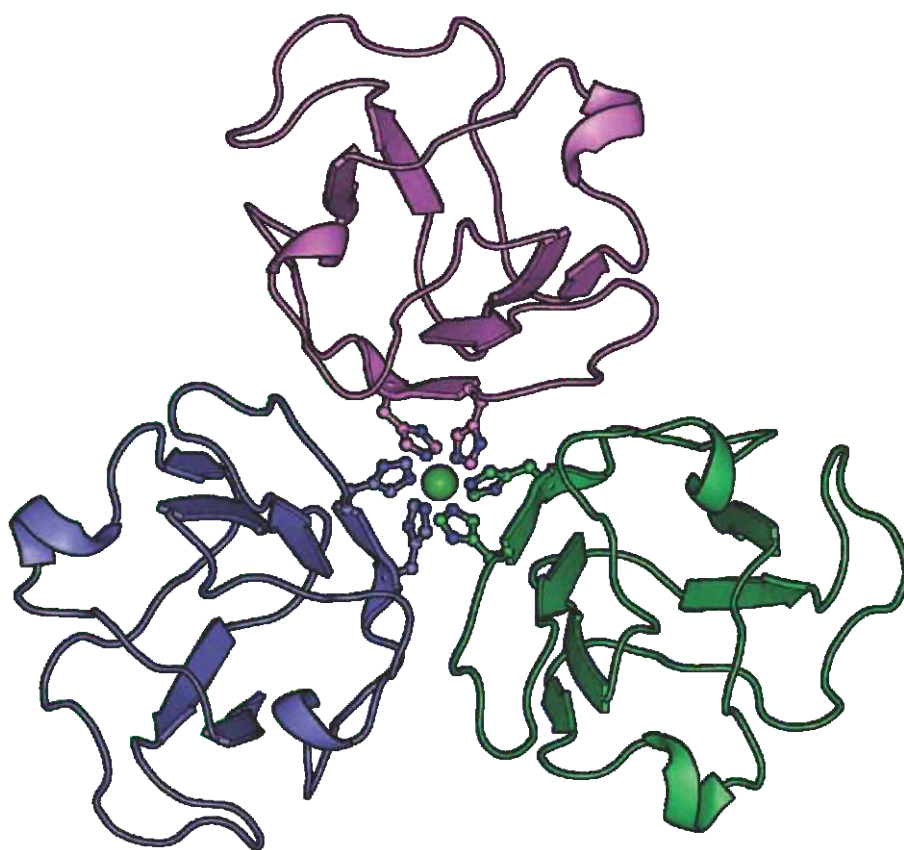
Pushpa Mitra Bhargava's vision for the Centre for Cellular and Molecular Biology is now the stuff of legend. CCMB was the idyll where about 20 of the finest minds led small- to medium-sized groups of scientists to investigate major unsolved questions in biology, and one could work any time of the day or night. The laboratories were centrally air-conditioned, and were equipped with walk-in cold rooms, emergency power supply, distilled water on tap, an extensive supply of fine chemicals and enzymes, a 24-hour centralized monitoring system. The famous artist Surya Prakash served as "Artist in Residence", and M. F. Hussain built his mural on the campus. Artists and sculptors camps were hosted and helped CCMB build up its own art collection, turning its lobby into a much admired art gallery.

Supporting staff had a sense of pride in the institution and truly felt they were essential to the success of an enterprise that was larger than them, and whose success would therefore be their achievement. Prizes won by cooks, drivers, gardeners, typists, and photographers were everybody's pride. Laboratory helpers were awarded much coveted certificates, later framed, for best maintained laboratories and office spaces. Everybody spoke as if CCMB belonged to them. The joke went that when Bhargava accompanied by two CCMB officers, V. K. Sarma and G. Kranti Kumar, walked into CSIR HQ, each spoke so passionately that they couldn't tell who was the Director.

Like many visionaries, Bhargava chose to overlook the flaws and limitations of those working for him. While some worried whether public money was being squandered, his magnanimity won him the abiding devotion and loyalty of its beneficiaries, and made the doubters look small-minded. He did not despair even as some research projects began to flounder, and the Nobel Prizes, so recklessly promised, did not materialize. Although these were taken by some as signals of the unraveling of his grand dream, one cannot fail to see the several spectacular successes that did derive from this dream, but I will not dwell on them here, except to say that many of the bright youngsters that he recruited paid back his confidence by publishing important research papers, winning national recognition through their awards, fellowships, and prizes, and some of them went to head universities and national laboratories.

On the personal level Bhargava's kindness and courtesy are unforgettable. He welcomed you with a warm smile and the seeming willingness to spend all day in your company. In his passing many of us lost a mentor and India lost a son who was greater than the sum of his many parts.

D P Kasbekar



CSIR - Centre for Cellular and Molecular Biology

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