United Nations
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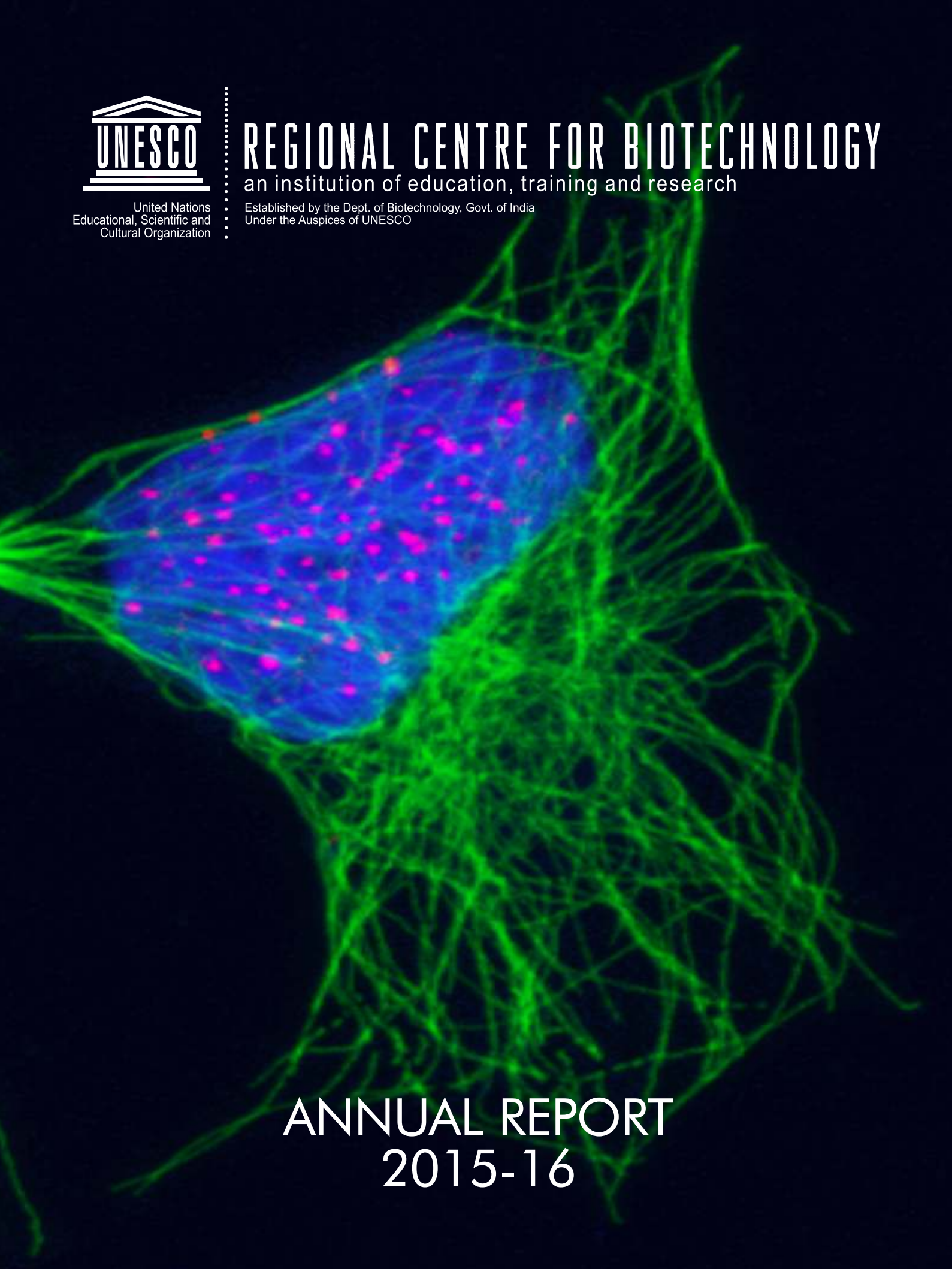
REGIONAL CENTRE FOR BIOTECHNOLOGY

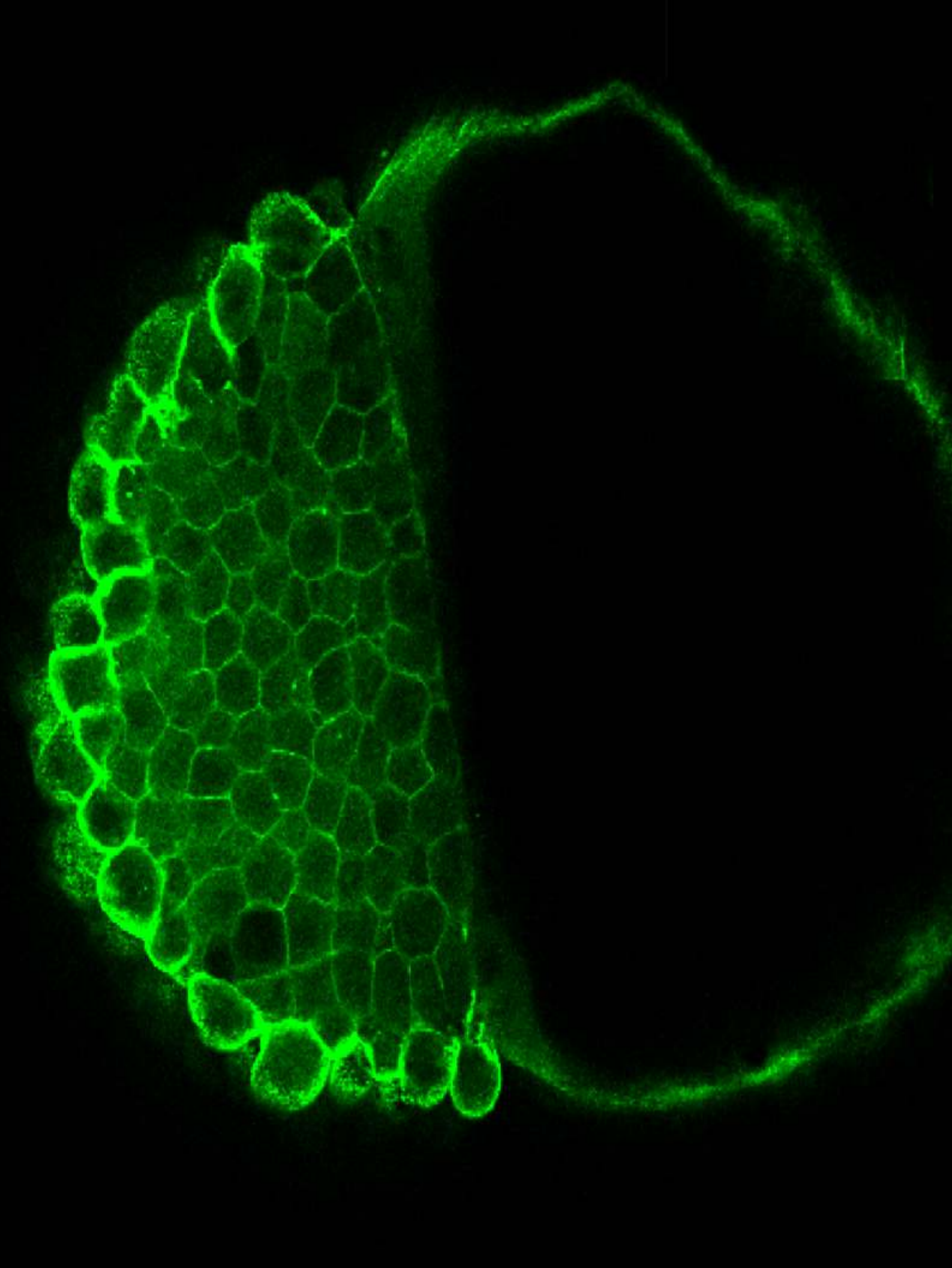
an institution of education, training and research

Established by the Dept. of Biotechnology, Govt. of India
Under the Auspices of UNESCO



ANNUAL REPORT
2015-16


RCB
REGIONAL CENTRE FOR BIOTECHNOLOGY



Contents

From the Executive Director's Desk

I am pleased to present the annual report of the Regional Centre for Biotechnology (RCB) for the period 2015-16, during which several important milestones were reached that would define the future of the Centre. The RCB was conferred the status of an institution of national importance through an Act of Parliament. This enables the Centre to grant degrees in education and research in biotechnology and related fields. To achieve its objectives, the Centre would discharge its functions in close collaboration with other national, regional and international institutions of UNESCO.

The Centre and its activities have continued to grow in the new campus. The year also saw the completion of the fourth wing of the RCB academic block, the Advanced Technology Platform Centre (ATPC) building, the student hostel and guest house, and the faculty housing. The completion of this phase of construction has created an additional laboratory and residential space for the future faculty and students of the Centre.

The Centre has continued to pursue the various innovative research programs to create knowledge in the broad areas of biotech science. Besides the various research areas pursued by the individual principal investigators, RCB continued to participate in a multi-institutional program to understand the biology of pre-term birth and identify possible biomarkers to predict the outcomes. A large cohort of pregnant women is currently being developed at the Gurgaon General Hospital and the RCB scientists are carrying out a systematic study on the proteome of the various tissue samples from these women.

The scientific reports section of the annual report provides details of the progress made under different programs pursued by the individual principal investigators. Some of the notable progress is highlighted below.

Under the research program on nanomaterials for biomedical applications, lipid-peptide conjugates forming hydrogels encapsulating different anticancer drugs in different combinations were developed. The study showed the anticancer potential of these drug-encapsulated hydrogels in the murine model systems. These hydrogels will now be explored for multi-targeting strategies aiming at cancer cell-intrinsic and cell-extrinsic pathways using anti-cancer, anti-inflammatory, and anti-angiogenic drugs.

NgoS is a mismatch repair protein of *Neisseria gonorrhoeae* that does not follow the classical E. coli mismatch repair mechanism. The studies on the assembly of the functional NgoS-DNA complex provide the structural transitions that enable the formation a protein toroid around DNA. This study provides the mechanism

utilized by NgoS to bend DNA to scan for mismatches and provides insight regarding the early events in DNA mismatch repair.

FleQ is the master regulator of flagellar genes in Pseudomonas aeruginosa and the antiactivator FleN is a putative ATP/GTP binding protein that interacts directly with it. Our studies have shown that ATP-induced structural remodelling facilitates the formation of the functional dimer in FleN and helps the anti-activator attain a reversible form that can calibrate FleQ activity to an optimum level. Knock-out of these proteins in Pseudomonas aeruginosa will now be used for the in vivo validation.

To derive gene regulatory networks underlying resistant and susceptible legume-powdery mildew interactions and identify novel pathways/genes associated with the interaction, an unbiased discovery based dual RNAseq of mRNAs and miRNAs has been performed. These data will now be used to identify the pathogen-secreted effector proteins.

Studies on platelet activation during dengue virus (DENV) infection show that high copy numbers of the virus genome in platelets directly correlated with platelet activation during the early infection. This was further supported by in vitro data showing the dose-dependent activation of platelet by DENV. DENV-mediated platelet activation was directly correlated with platelet lysis and clearance. Investigations are on to further understand the mechanism of rapid platelet depletion in dengue patients.

Inflammatory bowel disease (IBD) involves chronic inflammation of all or a part of the digestive tract leading to severe diarrheal, pain, fatigue and weight loss. Using cell culture and the mouse model, an important role for SUMOylation in inflammation during IBD and the colorectal cancer has been found, and these observations have been validated using patient samples.

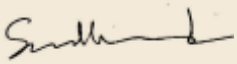
The academic activities at the RCB have continued with full vigour. Thirteen young scientists are currently mentored by the RCB faculty and there are 77 students pursuing doctoral research, of which 14 joined RCB during this academic session. In addition to this, a number of post-doctoral fellows and research fellows/assistants engaged through extramural grants are adding to the scientific strength of the Centre.

The UNESCO India cluster office and RCB organized a "Regional Dialogue on Science and Technology Policy in the context of Biotechnology towards Sustainable Development" during Dec 29-30, 2015. In the dialogue, eminent scientists, speakers, policy makers and academic institutions from more than 6 countries deliberated their country's S&T policy in the specific context of biotechnology. The Centre organized an international workshop on computational crystallography in partnership with the Collaborative Computational Project No. 4 (CCP4) executive of the United Kingdom. The workshop was attended by more than 60 scientists and researchers across the country and more than 15 national and international speakers mentored in their field of specialization.

RCB also hosted the Young Investigators Meeting (YIM-2016), a conference for post-doctoral fellows invited from international universities across the globe. The meeting serves as a platform for sharing the research experiences at the level of

post-PhD fellows and their opportunities to work on inter-institutional basis. In addition, RCB co-organized the Ramalingaswami Fellowship Conclave-2016 attended by more than 150 scientists and research fellows working in the pioneering areas of biotechnology. The conclave was mentored by 25 eminent scientists and speakers in their areas of expertise.

All of the above activities and others described elsewhere in this report were possible only due to the cooperation from colleagues from the RCB and THSTI administration and faculty, and support and encouragement from DBT and UNESCO, the members of the Board of Governors, the Program Advisory Committee, and the various other statutory committees, and I look forward to their continued support in the future.



Sudhanshu Vrati
Executive Director

Mandate of the Regional Centre for Biotechnology

The mandate of the Regional Centre for Biotechnology (RCB) is to provide a platform for biotechnology education, training and research at the interface of multiple disciplines. The programmes of the Centre are designed to create opportunities for students to engage in multi-disciplinary research where they learn biotech science while integrating engineering, medicine and natural sciences, to provide solutions for human and animal health, agriculture and environmental technologies.

The vision is to produce human resource tailored to drive innovation in biotechnology, particularly in areas of new opportunities and also to fill talent gaps in deficient areas. The Centre is regarded as a "Category 2 Centre" in terms of the principles and guidelines for the establishment and functioning of UNESCO Institutes and Centres.

The objectives of the Regional Centre are:

(a) to disseminate and to advance knowledge by providing instructional and research facilities in such branches of biotechnology and related fields as it may deem fit including technology policy development,

(b) to provide capacity-building through education, training, research and development in biotechnology and related academic fields for sustainable development objectives through regional and international cooperation,

(c) to facilitate transfer of knowledge and technology relating to biotechnology at the regional level,

(d) to create a hub of biotechnology expertise and to address human resources needs in the countries in the region,

(e) to promote and strengthen international co-operation to improve the social and economic conditions and welfare of the people,

(f) to promote and facilitate a network of satellite centres in the region as well as within India.

The functions of the Regional Centre are:

(a) to establish infrastructure and technology platforms which are directly relevant to biotechnology education, training and research,

(b) to execute educational and training activities including grant of degrees in education and research in biotechnology and related fields,

(c) to produce human resource tailored to drive innovation in biotechnology, particularly in areas of new opportunities and to fill talent gap in deficient areas,

(d) to undertake research and development and scientific investigations in collaboration with relevant research centre's in the region,

(e) to hold scientific symposia and conferences within India or in the region or outside the region and to conduct short-term and long-term training courses and workshops in all areas of biotechnology,

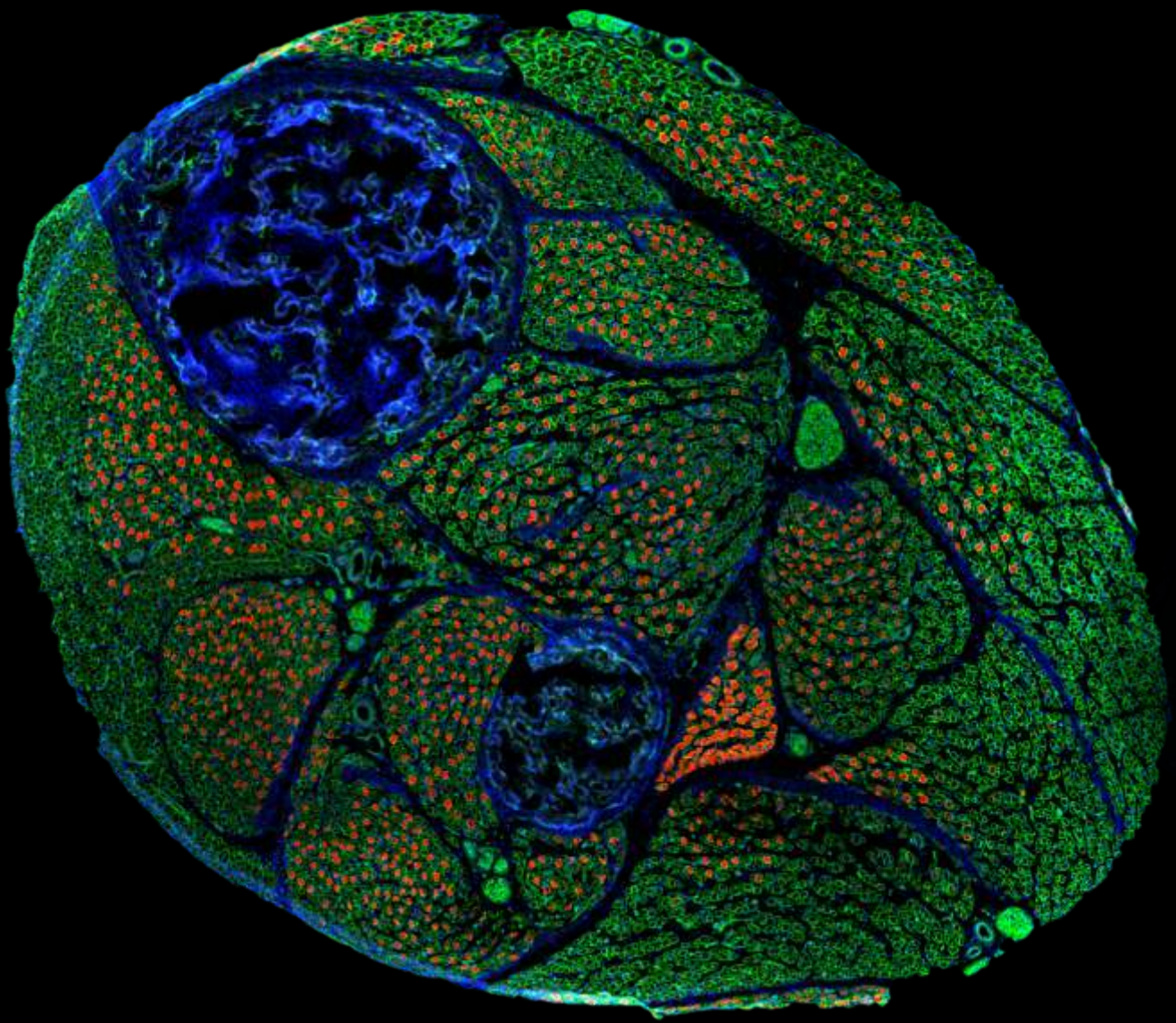
(f) to collect universally available information with a view to setting up data banks for bio-information,

(g) to collect and disseminate, through networking, the relevant local knowledge in the field of biotechnology, ensuring protection of intellectual property rights of local stakeholder communities,

(h) to develop and implement a policy for intellectual property rights which is equitable and just to the stakeholders involved in research in the Regional Centre,

(i) to disseminate the outcome of research activities in different countries through the publication of books and articles,

(j) to promote collaborative research and development networking programme in specific areas of biotechnology with national, regional and international networks and promote exchange of scientists, at the regional level having regard to issues pertaining to intellectual property rights of collaborating institutions promoting equitable sharing of benefits with collaborating institutions.



Scientific Reports

Progress during 2015-2016

7

Regional Centre for Biotechnology
an institution of education, training and research

Engineering of Nanomaterials for Biomedical Applications

Collaborators
Sagar Sengupta (NII)
Aasheesh Srivastava (IISER, Bhopal)

Ph. D. Students
Vedagopuram Sreekanth
Somanath Kundu
Kavita Yadav
Sandeep Kumar
Nihal Medatwal
Sanjay Pal
Animesh Kar
Priyanka Verma

Project Staff
Ruchi Tripathi

Avinash Bajaj
Principal Investigator

This research programme addresses the challenges in the area of membrane biophysics, cancer biology, and infectious diseases to develop nanomaterials for effective therapeutics for cancer and infectious disease using interdisciplinary approach of synthetic chemistry, cell biology, microbiology, and nanotechnology. Last year, bile acid-peptide based hydrogels was engineered for drug delivery applications.

Bile acids and amino acids are well-known bimolecular scaffolds that have the tendency to self-assemble and form supramolecular structures. Twenty lipidated-dipeptides (LD) were designed and engineered where C-terminal of dipeptides is glycine and N-terminal of dipeptides is one of the twenty naturally occurring amino acid. Dipeptides were conjugated to 3'-OH of benzylated lithocholic acid in step-wise peptide synthesis using HATU/HBTU chemistry; and were characterized using 1H-NMR and HRMS. Ability of each LD amphiphile to form hydrogel was evaluated using invert vial assay where different concentrations of LD amphiphiles were dissolved in water (pH 7.4) at 70 °C and allowed to cool at room temperature. Nine of the amphiphiles were able to self-assemble to form hydrogels with minimum gelation concentration of 50-65 mg/mL. Melting temperature (T_m) studies confirmed that Glycine-Glycine (LD-1) and Serine-Glycine (LD-12) derived LDs are most stable up to ~80 °C.

In vivo biocompatibility of LD-1 hydrogel was then characterized the in C57BL/6 mice, Sprague Dawley (SD) rats, and New Zealand (NZ) rabbits. Fluorescent (1% Rhodamine entrapped) LD-1 hydrogel was subcutaneously injected in adult C57BL/6 mice; and change in volume of the injected material was measured on alternate day. Intactness of LD-1hydrogel was observed till 20 days with half-life of ~15 days in mice, and its complete degradation on day 21. Similarly subcutaneous implantation of LD-1 hydrogel in SD rats confirmed the intactness of the gel for 22 days with half-life of ~14 days. Histology studies were then performed to evaluate the inflammatory response where tissue sections from mice implanted with LD-1 hydrogel were stained with haematoxylin and eosin, and no active infiltration of neutrophil or macrophage cells was detected. Sections were then stained with CD45 antibody to look for influx of CD45+ neutrophils. There was the influx of CD45+ cells in the subcutaneous tissues on day 10 that were absent on day 20 suggesting a transient influx of neutrophils that got cleared with time.

Study next evaluated the systematic toxicity of LD-1 hydrogel injection in C57BL/6 mice, SD rats, and NZ Rabbits by measuring the critical parameters like platelets, bilirubin, and liver enzymes like SGOT (Serum glutamic oxaloacetic transaminase) and SGPT (Serum glutamic-pyruvic transaminase). There was initial decrease in platelet counts on day 10 in mice that gets normalized on day 20. Increased SGOT and SGPT levels were observed after 20 days that were in

the normal range. In SD rats, all critical parameters were in normal range after 10 and 20 days with lower bilirubin levels on day 10; and normal levels of bilirubin, SGOT and SGPT after 90 days were observed in NZ Rabbits.

Having established bio compatible nature of LD-1 hydrogel, combination of CPT and DTX entrapped LD-1 hydrogel was evaluated for tumor regression studies in murine models. CPT was encapsulated into aqueous phase of hydrogels by dissolving it into LD-1 solution prior to hydrogel formation. LD-1 was able to retain CPT up to 8 mg/mL (~12% by weight). Drug release kinetics of CPT monitored by atomic absorption spectroscopy suggested the first order release profile. Similarly, LD-1 was able to entrap the DTX with high efficacy of 10 mg/mL (~15% by weight). Drug release kinetics using HPLC demonstrated small burst release (15%) of DTX followed by zero-order release that might be due to erosion-based phenomenon. LD-1 was also able to entrap combination of DTX and CPT at concentrations of 12 and 10 mg/mL; and CPT and DTX were able to maintain the first and zero order release profile respectively even when entrapped in combination.

Rheology studies were then performed to characterize the mechanical strength of LD-1hydrogel on entrapment of CPT (2.5 mg/mL), DTX (5.0 mg/mL) and their combination. Drug-entrapped LD-1 hydrogels were able to maintain the same mechanical strength; and elastic behavior of these gels was strengthened by 10- and 2-fold on entrapment of DTX and CPT respectively. LD-1hydrogel was able to efficiently entrap combination of hydrophobic (DTX) and hydrophilic (CPT) drugs as elasticity of the gel were strengthened by hydrophobic interactions of DTX with bile acid backbone and electrostatic interactions of CPT with peptide scaffold.

Inspired by dual-drug entrapment ability, and sequential and sustained drug release features of this chimeric hydrogel; effect of drug-entrapped hydrogels on tumor regression in two syngeneic murine cancer models was evaluated. In first case, study randomized 4T1 tumor bearing BALB/c mice into four different groups and subjected them to a) untreated control; b) single injection of DTX (50 mg/kg) and CPT (25 mg/kg) combination at tumor site (DTX-CPT-TS); c) ten intravenous doses of DTX (5 mg/kg) and CPT (2.5 mg/kg) formulation on alternate days (DTX-CPT-IV); d) single injection of DTX (50 mg/kg) and CPT (25 mg/kg) entrapped in LD-1 hydrogel at tumor site (DTX-CPT-Gel). Enhanced anti tumor effect was observed on DTX-CPT-Gel treatment followed by DTX-CPT-TS and DTX-CPT-IV treated group. No change in tumor regression was observed on LD-1 hydrogel (alone) treatment. No increase in mice survival was observed on DTX-CPT-TS and DTX-CPT-IV treatment over untreated control. DTX-CPT-Gel treated mice were observed to die after 55 days as compared to 45 days with mean survival increase of 6-days ($P < 0.0005$ vs. control). A significant decrease

in body weight (> 10%) of animals was observed on treatment with DTX-CPT-IV (P< 0.005 vs. control) whereas DTX-CPT-Gel treated mice were healthy. Study next compared the effect of DTX-CPT-Gel and DTX-CPT-TS on normal breast tissues in mice. Normal morphology of the breast tissue on implantation of DTX-CPT-Gel was observed, whereas enhanced tissue damage was present on DTX-CPT-TS treatment suggesting the sustained release of drugs from hydrogels is advantageous for normal tissues.

Next LLC tumor-bearing mice were subjected to similar treatments where similar tumor inhibition was observed on DTX-CPT-TS and DTX-CPT-Gel treatment. Significantly increased mean survival of the mice by 10 days (P< 0.005 vs. control) was observed on DTX-CPT-Gel treatment as compared to 4 days on DTX-CPT-TS treatment without any significant change in body weight. Study then measured proliferative activity in tumor samples using Ki67 immunostaining for nuclear protein Ki-67 that is a proliferative marker for carcinoma. Minimal proliferative activity was observed in DTX-CPT-Gel treated tumors as compared to DTX-CPT-TS and DTX-CPT-IV samples suggesting the maximum therapeutic effect of DTX-CPT-Gel. Effect of DTX-CPT-Gel on tumor vasculature and inflammation using CD31 and CD45 immunostaining was then investigated where CD31 stains the endothelial cells and CD45 helps in staining infiltrating neutrophils. Significant inhibition of tumor vasculature and minimal effect on neutrophil infiltration was observed on DTX-CPT-Gel treatment. Study then determined key toxicological parameters of LLC tumor bearing mice after different treatment regimes. Low hemoglobin and platelets with increased SGOT levels was observed in tumor bearing mice that were normalized on DTX-CPT-Gel treatment.

In summary, highly elastic injectable hydrogels were prepared utilizing the hydrophobicity of bile acids and hydrophilic character of peptides. Hydrogels were strengthened by non-covalent hydrophobic and electrostatic interactions making them injectable under increased strain conditions. Dual loading of hydrophobic and hydrophilic drugs and maintaining their sequential and differential release required for chemotherapy applications were maintained by these interactions. Single injection of these hydrogels was able to abolish the tumors without any toxicity and with substantial increase in mice survival. Therefore, a class of biocompatible injectable hydrogels with facile synthesis for cancer combination therapy applications has been demonstrated.

Collaborators
S. Vijaya (IISc)
Pradeepkumar PI (IIT-Bombay)
D. N. Rao (IISc)

Young Investigator
Vaibhav K. Pandya

Ph. D. Students
Rahul Sharma
Jithesh Kottur
Shivlee Nirwal
Naveen Narayanan
Deepankar Singh
Mary K. Johnson
Shilpi Nagpal
Minakshi Sharma
Asra Nasir Khan
Patterson Clement

Deepak T. Nair
Principal Investigator

Molecular Determinants of Genomic Integrity and Plasticity

The research theme of the laboratory involves molecules that either maintain genetic integrity or render genomic plasticity. The aim is to provide mechanistic insight into how organisms evolve and adapt to the environment.

For all cellular processes to function optimally, the integrity of the genome has to be maintained. Conversely, plasticity in the genome can relieve selection pressure imposed by an adverse environment. These two conflicting requirements have led to the presence of molecules and pathways that either prevent (e.g. DNA mismatch repair) or facilitate (e.g. error-prone Polymerases) changes in the genome. The antagonistic action of these two different sets of molecules ensure that genomic plasticity is calibrated to endow adaptive capability without severely compromising genetic viability. We aim to elucidate the structural mechanism utilized by different molecular determinants of genomic integrity and plasticity to achieve function.

With this broad aim in mind, the biological processes under scrutiny in the laboratory are (a) DNA Replication & Translesion DNA synthesis (b) Stress-Induced Mutagenesis (c) DNA Mismatch repair (d) Stress-induced epigenetic modification (d) Transposition and (e) Replication of the Japanese Encephalitis Virus genome. Our efforts will provide insight into how variation appears in the genotype and phenotype of organisms, especially in response to stress. The insight gained from these studies will also provide a robust platform for the development of novel therapeutic strategies against pathogenic bacteria and viruses. The progress made in some of these projects is described below.

Stress-Induced Mutagenesis: Low fidelity DNA polymerases (dPols), such as DNA polymerase IV (*Escherichia coli*) participate in stress-induced mutagenesis. The expression of these enzymes is up-regulated when *E. coli* encounters environmental and nutrient stress. Error-prone DNA synthesis by these dPols ultimately gives rise to multiple genomic templates for natural selection. This strategy allows microbes to adapt and relieve selection pressure imposed by an adverse environment. The mutator activity of DNA polymerase IV (PolIV) is predicted to exist within an appropriate range, as too many mutations will compromise genetic viability and too few may not provide adequate genotypes for natural selection. Since the presence of antimicrobial agents in the environment is a source of great stress for prokaryotes, the activity of low-fidelity dPols such as PolIV can lead to the appearance of drug resistant strains in pathogenic bacteria.

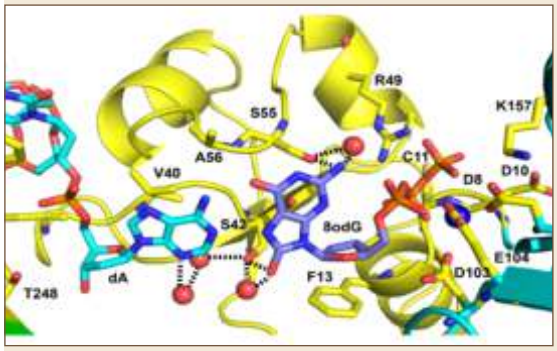


Fig. 1. Structure of PolIV in complex with DNA and 8oxodGTP (8odG).

Through studies on PolIV, we aim to shed light on how these enzymes ensure adequate sampling of the mutation space and thus enhance our knowledge of how organisms evolve and adapt. We have previously discovered an important attribute of the PolIV active site that is important for the ability of this enzyme to promote substitution mutations (Sharma et al, 2013, Nucleic Acids Research, 41:5104). PolIV is known to extend from mismatches and also promote frame shift mutations and at present, efforts are underway to understand the structural basis of these activities of PolIV.

Recent studies posit that reactive oxygen species (ROS) contribute to the cell lethality of bactericidal antibiotics. However, this conjecture has been challenged and remains controversial. To resolve this controversy, we adopted a strategy that involves PolIV. The nucleotide pool of the cell gets oxidized by ROS and PolIV incorporates the damaged nucleotides (especially 8oxodGTP) into the genome, which results in death of the bacteria. Using a combination of structural (Fig. 1) and biochemical tools coupled with growth assays, we have shown that selective perturbation of the 8oxodGTP incorporation activity of PolIV results in considerable enhancement of the survival of bacteria in the presence of the norfloxacin antibiotic (Kottur & Nair, 2016, Angewandte Chemie, 55:2397). These studies therefore indicate that ROS induced in bacteria by the presence of antibiotics in the environment contribute significantly to cell lethality.

DNA Mismatch Repair: The Mismatch Repair (MMR) Pathway serves to maintain genomic integrity by correcting errors that appear during replication. In *E. coli*, the specific components of MMR are MutS, MutL and MutH. A majority of bacteria and all eukaryotes lack a homolog of MutH. It is, therefore, expected that these organisms will show significant differences in MMR especially in the mechanism of strand discrimination and nick-creation. Using the pathway from *Neisseria gonorrhoeae* as a model system, the aim is to elucidate the mechanism of MMR in organisms that do not follow the *E. coli* paradigm. The MutS and MutL homologs in *Neisseria* are named NgoS and NgoL, respectively. MutS represents the primary mismatch sensor and forms a dimer clamp that encircles DNA and bends it to scan for mismatches. The mechanism by which the MutS dimer encircles DNA is not known, and the origin of force required to bend DNA is unclear. Additionally, there is limited knowledge regarding the dynamics of ATP binding to MutS during the process of DNA loading. We have elucidated the mechanism of assembly of the functional NgoS-DNA complex. Our study shows that, in the absence of DNA, there exists a gap between the monomers through which DNA can enter the central tunnel (Fig. 2). One of the monomers will then move

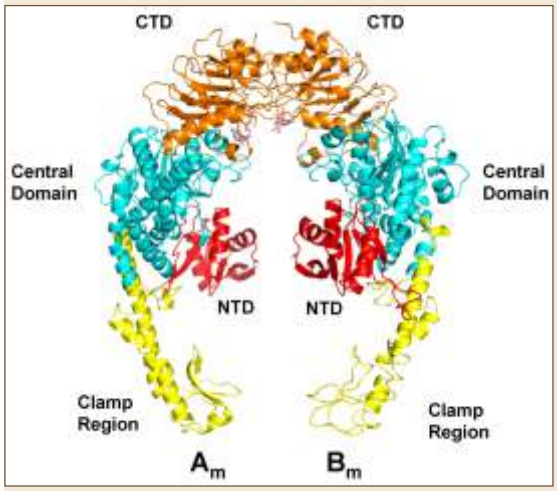


Fig. 2. Structure of the $NgoS_{ADP}$ complex.

by nearly 50 Å towards the other monomer and the clamp regions associate to complete formation of a toroid around DNA. The structural inferences are supported by DLS studies that show that the NgoS molecule undergoes compaction in the presence of DNA. Due to this structural transition, the N-terminal domains of both monomers press onto DNA to bend it. The MutS dimer, therefore, acts like a pair of pliers to bend DNA. The movement of the monomer is facilitated by the C-terminal region which acts as a hinge and also connects monomer movement to structural changes in the ATP binding site that result in the expulsion of ATP from the fixed monomer. Overall, the study shows that structural transitions on DNA binding enable formation of the dimer clamp and bending of DNA and provides mechanistic insight regarding the early events in DNA Mismatch Repair.

Stress-induced epigenetic modification: There are an abundance of restriction modification (RM) systems in the gastric pathogen *Helicobacter pylori*. These RM systems regulate natural transformation and consequent genomic plasticity of this microbe. Some DNA Methyltransferases (dMtases) that are part of these RM systems, exhibit activity only in adverse conditions. Methylation of cognate sequences by these enzymes results in the alteration of transcriptional profiles to rapidly respond to stress in the environment. The aim is to elucidate the regulatory mechanisms that permit these enzymes to act only under specific environmental conditions.

The expression of the HP0593 dMtase is upregulated when the pathogen encounters low pH. HP0593 is a TypeIII dMtase that belongs to the β class of these enzymes. The enzyme exhibits optimal activity at pH=5.5 and is predicted to modulate the expression of different genes to relieve acid stress. We have determined the structure of HP0593 in complex with the inhibitor sinefungin (SFG).

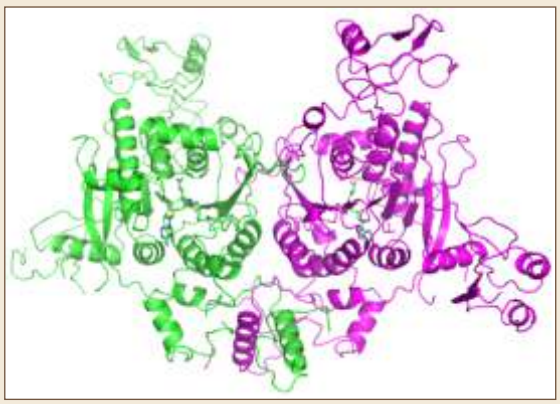


Fig. 3. Structure of HP0593:SFG complex.

The structure (Fig. 3) coupled with biochemical and biophysical analysis of site-specific mutants of HP0593 suggests that a functional oligomer of this enzyme is formed only at low pH. This property of HP0593 ensures that it is licensed to act only when the organism is subjected to acid stress.

Young Investigator
Amit Yadav

Ph. D. Students
Chanchal
Priyajit Banerjee
Swatee Upadhyaya
Vaishali Uniyal

Research Associate
Mrittika Sengupta

Project JRF
Pankaj Kumar Sahoo

Deepti Jain
Principal Investigator

Transcription Regulation: Structure and Mechanism

The research in the laboratory involves structural studies of macromolecular complexes involved in regulation of bacterial gene expression. The ongoing projects are focused on elucidating the mechanism utilized by bacterial enhancer binding proteins and their associated factors to regulated transcription, understanding the mechanism utilized by single subunit mitochondrial RNA polymerase for initiation of synthesis and elongation of the mRNA transcripts, addressing the allosteric mechanism that renders transcription factors responsive towards small metabolites and elucidating the mechanism of stress-induced crosstalk between two component systems.

Bacterial enhancer binding proteins belong to AAA+ (ATPase associated with various cellular activities) family of proteins that typically form oligomers and utilize the energy from ATP hydrolysis to remodel their substrates. All bEBPs, like the eukaryotic transcriptional activators have a modular structure consisting of three distinct functional domains. The N- terminus domain serves as a target for regulatory signals. The C-terminus DNA binding domain consists of a helix-loop-helix motif and recognizes the upstream activating sequences. Although structures of various domains of 54-dependent activators and cryo-electron microscopy reconstruction of one of the activator bound to RNAP is available, the information on nature of conformational changes that occur in the activator on RNAP binding is lacking. Most importantly, the allosteric mechanism that results in melting of promoter DNA bound to 54-RNAP on ATP hydrolysis by bEBPs is not known. In order to answer these questions, FleQ, a master regulator of flagellar genes in Pseudomonas *aeruginosa (Psa)* has been employed as a model system. FleQcontrols the expression of flagellar genes in a 54 dependent fashion and is present at the apex of the flagellar transcription cascade. Crystallization trials of constructs with different domains and domain combinations alone or in complex with ATP or DNA are currently ongoing. The activity of FleQ is in turn regulated by another protein FleN. FleN is a putative ATP/GTP binding protein that interacts directly with FleQ without affecting its DNA binding ability. Thus, FleN acts as an antiactivator that regulates flagellar numbers by regulating the activity of FleQ. In order to obtain mechanistic insights into the modes of transcription regulation by FleN, the molecular interactions between FleQ and FleN are being investigated. To discern the functional form of FleN that regulates FleQ, the structure of FleN alone (1.66 Å) and bound to non-hydrolysable ATP analog, β,γ-Imidoadenosine 5-triphosphate–AMPPNP (1.55 Å) have been determined. Comparison of these two high resolution structures reveals that FleN undergoes

drastic conformational changes on ATP binding that enables the formation of a stable dimer. Site specific mutants of FleN that perturb the ATP binding result in proteins that are poor inhibitors of ATPase activity of FleQ. These mutants are incapable of forming dimers in solution due to reduced affinity for ATP. However, the mutation that abolishes ATPase activity of FleN gives rise to a better inhibitor of ATPase activity of FleQ. This indicates that the ATP hydrolysis by FleN is dispensable for antagonistic effect against FleQ and dimerization of FleN is essential for its anti-activator effect. Overall the studies reveal that ATP-induced structural remodeling facilitates formation of the functional dimer in FleN and helps the anti-activator attain a reversible form that can calibrate FleQ activity to an optimal level. Currently validation of the structural studies *in vivo* are ongoing through complementation assays using the knockout of these proteins in *Pseudomonas aeruginosa*.

Another model system being utilized to get atomic level information on transcription is the single subunit RNA polymerase from mitochondria. These enzymes represent a distinct class of DNA dependent RNAPs that are involved in transcription of small genomes such as those found in phages and organelles of eukaryotes namely mitochondria and plastids. Interestingly, mitochondria and plastids possess their own genomes and transcription machineries. The mitochondrial transcription in A. *thaliana* is mediated by two single-subunit (~100KDa) RNA polymerases – RpoTm and RpoTmp. RpoTm is vital for plant development and serves as the basic RNAP in mitochondria. RpoTmp on the other hand transcribes a subset of mitochondrial genes that are not defined by a common promoter sequence. RpoTm is known to initiate transcription from different mitochondrial promoters but the structural basis of this ability is unknown. The enzyme recognizes diverse DNA sequences, melts the DNA to generate the transcription bubble (initiation phase) and extends the elongating mRNA (elongation phase). Mechanistic details at each of these stages are not known for RpoTm. AtRPoTm is a 976 amino acid containing protein. At the N terminus there is a mitochondrial targeting peptide of 42 amino acids. AtRPoTm has two domains the N terminal domain and a C terminal domain. The enzyme has been cloned expressed and purified and is capable of performing transcription *in vitro* whereas the catalytic mutant is inactive. On the basis of structure prediction, a deletion construct of the enzyme which lacks 56 residues from the N-terminus has been constructed. This new constructs gives better yield as compared to wild type enzyme. The deletion construct is also catalytically active in *in vitro* assays. Crystallization trials are underway for initiation and elongation complex with both the full length enzyme and the deletion construct.

Allostery has been defined as the fundamental process wherein the binding of a ligand or the effector molecule alters the activity of the protein at a distant site. In case of transcription modulators, effector binding can either increase the affinity (activation) or can decrease its affinity to the DNA (derepression) thereby altering the gene expression. Thus, transcription modulators serve as molecular switches, turning on and off the expression of genes. AraR protein is the key regulatory protein of the L-arabinose metabolism in *Bacillussubtilis*. AraR is composed of two independent domains exhibiting different functions and belong to different family of proteins. The smaller N-terminus domain (NTD), which retains its ability to bind DNA, comprises winged helix-turn-helix motif and the larger C-terminus domain (CTD) binds L-arabinose and belongs to LacI/GalR family. In the absence of L-arabinose, AraR binds to operator sequences and suppresses the expression of metabolic genes. Presumably, AraR undergoes a conformational change on binding L-arabinose which releases it from cognate operators resulting in transcription initiation. The crystal structures of C-terminal domain of AraR in complex with L-arabinose and NTD in complex with four different operators are known. The full length AraR in complex with L-arabinose has been crystallized and X-ray diffraction data was collected at 3.2 Å resolution. Currently, structure solution is in progress and efforts are underway to obtain the crystals of full length AraR bound to DNA. The data will provide insights into the structural basis for the allosteric mechanism in AraR that abolishes specific DNA recognition on arabinose binding.

The research program in the lab also addresses how the environmental signals are perceived by the bacteria. The two component systems are signaling pathways that enable bacteria to sense and respond to diverse ranges of environmental signals such as pH, nutrients, antibiotic stress etc. A typical two component system comprises of a sensor histidine kinase (S) and

a cognate response regulator (R). The histidine kinase receives the signal that activates the system, whereas the response regulator is often a DNA binding transcriptional regulator. The activation domain of histidine and the receiver domain of the response regulator are widely conserved as a result the histidine kinase of one regulatory system can sometimes, activate the regulator of another. This phenomenon is known as "cross-talking of two component systems.

The VraSR (vancomycin resistance associated) system is a typical two component system in *Staphylococcus aureus*, where VraS is the sensor histidine kinase and VraR is the response regulator. It was observed earlier that the inactivation of VraS, resulted in increased tolerance to glycopeptide antibiotics like vancomycin raising a possibility that the response regulator was being activated by an alternative kinase possibly GraS. GraS is part of the GraSR (glycopeptide resistance associated) two component system. Rigorous structural investigation of the interactions responsible for cross talk between the VraSR and GraSR regulon is the aim of this project. Particularly, the role of GraX in communicating the signals for cross talk between VraSR and GraSR regulon are under scrutiny. The ΔgraX knockout mutant strain of *Stahas* been prepared. As expected the mutant *Sta*was more sensitive to antibiotics like vancomycin and oxacillin. In order to verify if GraX is the potential candidate for mediating the cross talk between the two two-component systems, the effect of this mutation has been analysed using qRT-PCR with genes under both VraSR and GraSR regulons. The data shows that deletion of graX affects both the VraSR and GraSR genes. The gene expression analysis in presence of vancomycin stress shows that the genes involved in the cell wall metabolism under both the regulons were down regulated. In addition it was observed that the graX mutant was more prone to forming biofilms as compared to *wt*Sta. The role of VraSR and other two component systems in regulation of biofilm formation in *Sta* will be investigated. Structural characterization of these interactions by co-crystallizing the relevant proteins in order to understand the mechanism of the "Cross-talk" between the two two-component systems is underway. Antibiotic resistance in *S. aureusis* one of the leading causes of mortality and healthcare expenditure. Thus understanding the complex regulatory networks mediating such resistance is very important. This work will aid in deciphering the putative network that is responsible for the increase in glycopeptide tolerance in *Sta*.

Modulation of Host Immunity and Nutrient Allocation by a Biotrophic Pathogen

Young Investigator
Rashi Gupta

Ph. D. Students
Megha Gupta
Arunima Gupta
Akriti Sharma
Pankaj Kumar

Postdoctoral Fellow
Gunjan Sharma (SERB National PDF)

Divya Chandran
Principal Investigator

The research programme explores how the obligate biotrophic powdery mildew pathogen manipulates living plants to acquire nutrients while restricting defense responses. The study aims to elucidate the molecular, cellular and genetic basis of host immunity as well as susceptibility by studying the interactions between the powdery mildew pathogen *Erysiphe pisi* and its legume hosts, *Medicago truncatula* and pea.

The major goal of the research is to identify host processes and process components mediating powdery mildew (PM) resistance as well as susceptibility factors that are required for sustained biotrophic growth in legume-PM interactions. Concurrently, the aim is also to understand how the obligate biotroph modulates host processes for its benefit. It is anticipated that targeting a combination of such factors would result in dramatically reduced pathogen proliferation and contribute to durable resistance that is less likely to be rapidly overcome by pathogen counter-evolution. The following major objectives will be pursued using the model pathosystems: 1.Identification of novel infection site-specifichost processes and process components impacting PM proliferation. 2. Investigation of mechanisms underlying basal defense and effector-triggered immunity (ETI) in host-PM interactions.3.Elucidation of key factors mediating carbon (re)allocation at the host-biotroph interface.4.Identification of PM effector candidates and their host targets. Powdery mildew is a significant disease of legumes, which represent important food crops consumed in India and other developing countries. PM fungi are obligate biotrophic pathogens that can only propagate on living plant cells. They modulate host cellular architecture and metabolism to divert nutrients to fuel their own growth and reproduction while limiting host defense responses. The disease appears as a characteristic white powder on the surface of leaves, stems, and fruits. PM fungi form long-term feeding relationships with their hosts via the formation of specialized organs termed haustoria within host epidermal cells. Development of the haustorium, which is required for nutrient and water uptake by the fungus, occurs by 24 hours post inoculation (hpi). Further growth results in the formation of surficial hyphae and secondary haustoria, with asexual reproductive structures (conidiophores w/conidia) formed by 5 dpi. At ~7 dpi, the fungus is clearly visible as a white powder on the host surface. Wind-dispersed conidia rapidly propagate new infections. Current chemical methods used to control the disease are neither economical nor environmentally sustainable. To develop innovative

biotechnological strategies to control the disease, it is important to elucidate the mechanisms underlying legume-PM interactions.

The model legume *Medicago truncatula* is a valuable resource for understanding disease biology in legumes owing to its small diploid sequenced genome, high degree of synteny/co-linearity with important food legumes, and availability of PM resistant and susceptible genotypes and mutant populations for reverse genetics studies. *M. truncatula* accessions exhibiting varying degrees of resistance/susceptibility to an Indian isolate of the PM pathogen *Erysiphe pisi* were previously identified by other labs. The highly resistant genotype identified in this study was previously shown to be similarly highly resistant to other isolates of the pathogen. Genetic studies have so far identified four resistance loci that contribute to isolate-specific PM resistance in this genotype. For 3 resistance loci, the exact identity of the genes conferring resistance have not been determined. However, for the fourth one, resistance was narrowed down to a single gene encoding an R protein. R proteins contain nucleotide-binding and leucine rich repeat domains (NB–LRR) and play important roles in effector-triggered immunity (ETI). ETI is the second line of defense in plants where direct or indirect recognition of pathogen virulence factors (effectors) by plant R proteins stimulates generation of ROS, induction of the plant defense hormone salicylic acid (SA) and massive transcriptional reprogramming of defense-related genes, often culminating in a hypersensitive response (HR). A microscopic evaluation of *E. pisi* isolate Tanda growth on the highly resistant *M. truncatula* genotype showed that *E. pisi* growth is arrested prior to haustorium formation. Further, preliminary assays showed that hydrogen peroxide and SA accumulated in infected leaves at early phases of the interaction, consistent with an early R gene-mediated oxidative burst and defense induction. Molecular and genetic tools are currently being employed to identify regulators of this resistance response.

Host responses to PM infection are typically localized to a few cells surrounding the fungal haustorium-containing plant cell. Laser capture micro dissection (LCM) coupled with next generation sequencing is currently being employed to enhance sensitivity at detecting novel host genes and pathogen effectors with altered expression at the infection. For LCM, plant tissues have to be optimally fixed to preserve host cell morphology (for identification of infection sites) and RNA integrity (for downstream expression profiling). Three chemical fixatives, namely farmer's fixative, methanol and acetone were compared using a paraffin method for preparation of *E. pisi*-infected and uninfected *Medicago* leaf tissues. Highest preservation of leaf internal structure was observed with farmer's fixative

and methanol. Further, fungal haustoria were clearly visible within epidermal cells of infected leaf sections. RNA yield, purity and integrity were also checked at several stages of tissue preparation. It was found that RNA yield and purity were optimal at all stages of tissue preparation except after paraffin embedding. Currently, RNA stabilization solutions are being incorporated during this step to check whether they can preserve RNA integrity.

Biotrophic pathogens like the PM fungi acquire nutrients, especially sugars, from their host through haustoria. These structures create an apoplastic interface between the host and the fungus, through which released host nutrients are absorbed by the pathogen. As a consequence, a source-to-sink transition is triggered in infected host tissues, modifying sugar transport and carbon partitioning at the whole plant level. To identify sugar transporters with a role in providing nutrients to the PM pathogen, the plant SWEET family of sugar transporters was first examined. SWEETs (Sugar will eventually be exported transporters) are a family of sugar efflux transporters that have been recently implicated in pathogen nutrition. For example, bacterial pathogens of the *Xanthomonas* species that cause bacterial blight disease in rice co-opt plant SWEET transporters to access host sugar reserves. Notably, when SWEET function is blocked, the growth and virulence of the pathogen is also reduced. It has recently emerged that in addition to the *Xanthomonads* other bacterial and fungal pathogens (including PM) modulate the expression of plant *SWEET* genes. Using a bio-informatics approach, 24 SWEET candidates were identified in *M. truncatula*, of which 13 were detected in leaf tissues. Expression patterns of these 13 candidates were then tested at different time points after *E. pisi* infection using quantitative real-time PCR (qPCR). Prior to *MtSWEET* expression analysis, fungal infection of leaf samples used for the analysis was validated by checking expression of a fungal-specific proton ATPase gene *(PMA)* and a host specific marker of the infection process, *Pathogenesis-related protein 10* (*PR10*). As expected, *EpPMA* expression was detected only in infected samples whereas *PR10* expression was up-regulated in infected samples. qPCR analysis showed that expression of most *MtSWEETs* was either unaltered or significantly down-regulated upon *E. pisi* infection at all time points post infection. Extending the whole leaf expression analysis for *MtSWEETs*, expression of these genes will be investigated at the infection site using LCM. Further, the role of MtSWEET and/or other sugar transporter candidates will be functionally characterized via reverse genetics approaches.

To derive gene regulatory networks underlying resistant and susceptible legume-PM interactions and identify novel pathways/genes associated with the interaction we performed an unbiased, discovery based dual RNASeq of mRNAs and microRNAs (miRNAs). miRNAs are small RNA molecules (~22 nt) that mediate gene regulation by interacting with target mRNAs and inhibiting translation and/or initiating cleavage of the target mRNAs. miRNAs have been shown to regulate gene expression in plants in response to several abiotic and biotic stresses including PM infection. Global mRNA and miRNA expression profiles of two *M. truncatula* genotypes with contrasting PM resistance phenotypes were generated to enable the dissection of host defense responses from susceptibility factors. Furthermore, an early infection time point (1 dpi) was selected based on the assumption that host defense responses would still be active in the resistant (R) genotype while nutrition acquisition would have initiated in the susceptible (S) genotype. Prior to sample submission, microscopic evaluation and quantification of pathogen growth was performed on both genotypes. At 1 dpi, growth of *E. pisi* Tanda was arrested at the multi-lobed appressorium stage on the R genotype whereas the pathogen was able to form primary haustorium and secondary hypha on the S genotype. In addition, fungal infection of leaf samples was validated using fungal and host specific markers of infection. A 6-fold higher expression of *EpPMA* was observed in infected S samples compared to R at 1 dpi. Conversely, expression of the host defense marker *PR10* was higher in the R genotype compared to S. To estimate the depth of sequencing required to profile both host and fungal transcriptomes, relative amounts of host and fungal ribosomal RNA (rRNA) were estimated in the two samples using qPCR. In infected R samples, fungal and host rRNA abundance was ~2% and 98%, respectively whereas in infected S samples, fungal and host rRNA abundance was ~5% and 95% respectively. Based on these results, paired-end deep sequencing was performed using the Illumina HiSeq platform and this data is currently being analyzed.

Pathophysiology of Thrombosis, Inflammation and Immune Responses in Different Disease Conditions

Collaborators
Tulika Seth (AIIMS)
Naval K Vikram (AIIMS)
Parvaiz Kaul (SKIMS)
Subodh Kumar, (AIIMS Trauma Centre)
JC Suri (Safdarjang Hospital)
MK Sen (Safdarjang Hospital)
Satyajit Rath (NII)
Vineeta Bal (NII)
Shinjini Bhatnagar (THSTI)
Sudhanshu Vrati (THSTI)
P Thigarajan (Baylor College of Medicine)
Yuandong Peng (Baylor College of Medicine)
Josef T Prchal (University of Utah)

Young Investigator
Sheetal Chawla

Ph. D. Students
Amrita Ojha
Angika Bhasym
Gowtham K Annarapu
Rashi Singhal
Sulagna Bhattacharya
Nishith Shrimali
Saibal Saha

Prasenjit Guchhait
Principal Investigator

The research programme is investigating the complex pathophysiology of pro-coagulant states, thrombosis and inflammations and immune functions in different disease and stress conditions. *Current research projects* aim to elucidate the following mechanisms of: 1.regulation of thrombo-inflammatory complications by intravascular hemolysis in diseases such asparoxysmal nocturnal hemoglobinuria (PNH), aplastic anemia (AA), sickle cell disease (SCD) and hemolytic uremic syndrome (HUS); 2. development of systemic inflammation in accidental trauma victims: role of intravascular hemolysis; 3.hypobaric hypoxia regulation of coagulo-inflammatory complications and immune responses of individuals at high altitude; 4. pathophysiology of thrombocytopenia in dengue infections; 5.complement factor H (CFH)-related protein 1 (CFHR1) and its role in maintenance of immunological tolerance; and 6.developmental and functional properties of the neonatal immune system.

Recent study described that Hb binding to glycoprotein 1bA on platelet surface induced platelet activation and apoptosis in a concentration-dependent manner, and promoted pro-thrombotic complications. Data from PNH patients showed the significant correlation among intravascular hemolysis, platelet activation and pro-thrombotic events, Singhal *et al*. 2015. Study has shown that Hb stimulated the platelet thrombus formation under physiological flow shear stresses simulating arterial and venous blood flow, Annarapu *et al*. 2016. Furthermore we have shown that Hb bound to an active site of von Willebrandfactor (VWF) under high share stress and enhanced thrombus formation, as observed in ECMO dialysis device *in vitro*, Da *et al*. 2015. Study has described that the mutant subtype of Hb, sickle Hb (HbS) bound to platelet and enhanced the platelet thrombus formation. HbS-mediated platelet activation correlated significantly with pro-thrombotic markers in sickle patients, Annarapu *et al*. 2016 (in communication). Study also have investigated the thrombo-inflammatory role of free Hb. Study observed that upon the intake of Hb-activated platelets the classical monocytes (CD14) were transformed in large numbers into inflammatory lineage ($CD16^{high}$). The $CD16^{high}$ cells were highly thrombogenic. These cells showed rapid cell death releasing high amount of pro-thrombotic/inflammatory factors into extracellular space. The in vitro observations are further supported by data from PNH and sickle cell patients,

Singhal *et al*. 2016 (in communication). We also investigated the differentiation of monocytes under hemolytic conditions. We observed that upon intake of Hb-activated platelets the THP1 cells were differentiated rapidly within 24 hours (normal cells took 48hr) into mature M1 macrophages (pro-inflammatory) subtype. The M1 macrophages showed impaired phagocytosis, acquired necrosis with in 48 hours releasing significant amount of pro-inflammatory cytokines and tissue factor. Likewise, the primary monocytes also displayed faster differentiated into M1 showing altered phenotypes and functions when engulfed Hb-activated platelets, Chawla *et al*. 2016 (manuscript in preparation). Furthermore we are also investigating the role of intravascular hemolysis in various aspects of innate and adaptive immune responses in patients with AA and PNH.

While investigating the effects of Hb on monocyte and neutrophil study observed the hyper-activation of these cells and in turn release of pro-inflammatory cytokines *in vitro*. Study also observed that the supernatant of these treated cells activated further the freshly isolated counterparts and other immune cells including dendritic cells, T and B-lymphocytes *in vitro*. Since the mechanical injury releases huge amount of cell free Hb into extravascular space at the site of injury in trauma patient, we decided to investigate the effects of Hb on the phenotype and functions of neutrophils and monocytes from the site of injury. Also measuring the cytokines released by these inflammatory cells and also the free Hb at extravascular space and also intracellular Hb in these cells. The final focus to investigate- how the persistent inflammations at the site of injury in some patients, develops into a systemic inflammations: whether free Hb and/or platelet factors are contributing to the above mechanisms.

As an extension of the recent findings of novel mutations in hypoxia responsive gene *EGLN-1* (C12G and G380C) and their association with the decreased erythropoiesis in native Tibetans at high altitude [Lorenzo et al. 2014, Tashi*et al*. 2016 (in communication)], study is now investigating the association of these polymorphisms with immune cytome of the native highland (Leh) vs. lowland (Delhi) Tibetans with and without the above mutations. *In vitro* studies are in the process of generating cell lines (THP1 or U937) with the above mutations using lentiviral expression system by knocking out first the endogenous gene and putting in the mutant constructs. Using the mutant and wildtype EGLN-1 cells we will investigate the role of prolyl hydroxylase [PHD2, negative regulator of hypoxia inducible factor (HIF)1α] in differentiation and immune functions of these cells in response to hypoxia. Using the *in vivo* and *in vitro* approach we

focus to investigate the role of the novel mutations of *EGLN-1* (C12G and G380C) in the adaptation and immune response of the Tibetans to infections and also to adverse clinical events such as edema and inflammation. The work is in progress. Also study is investigating: 1) thrombotic and coagulation markers in highland vs. lowland Tibetan with and without mutation in *EGLN-1* (C12G and G380C); 2) highlanders (Tibetans and non-Tibetans) vs. sojourners (reside temporarily). Also we are investigating the mechanism of hypoxia regulation of thrombo-coagulatory pathways, specifically regulation of EGR-1, which induces tissue factor synthesis, and PAI-1, which regulates plasmin synthesis by EGLN-1-HIF-1α axis or independent of EGLN-1-HIF-1α axis using gene manipulation in THP1 or K562 cells.

Studies have described the mechanism that the activation status of platelet determined the lysis or clearance of platelets. Study has shown that high copy numbers of dengue virus (DENV) genome in platelets directly correlated with platelet activation during early day (day 4) of fever and decreased during day 10. The complement-antibody mediated platelet lysis and clearance was maximum during day 4/6 of fever. The above observation was further supported by *in vitro* data showing the concentration-dependent activation of platelet by DENV. The DENV-mediated platelet activation was directly correlated with platelet lysis and clearance. Interestingly the DENV-mediated platelet activation and lysis was abrogated in presence of platelet activation inhibitor such as prostacyclin, Ojha *et al*. 2016 (in communication). Study is investigating further the mechanism of rapid replication and propagation of DENV in platelet and in other immune cells in dengue patients.

It is a very unique phenomenon that the homozygous deletion of CFHR-1 (complement factor H related1) gene (CFHR-1-/-) exists in 90% patients with atypical hemolytic uremic syndrome (aHUS) and also in 8-10% healthy individuals (irrespective of Caucasian or Indian). Reports also suggest that approximately 60% of aHUS patients with CFHR-1-/- have the high antibody titer for CFH (complement factor H), which is one of the major regulator of the alternative pathway of complement system, exits in serum as well as on cell surface. Data describe that healthy

individuals with CFHR-1-/- genotype have less ratio of myeloid dendritic cells (DC)/plasmacytoid DC and high number of plasma blast cells along with high binding of CFH on classical monocytes (CD14+CD16-). Our data also show that the activated (CD25+ve) CD4 T cell proportion is higher in CFHR-1-/- individuals. Furthermore we are analyzing the immune subsets of CD4 T cells that are Th1, Th2, Th17 and T regulatory cells (induced or natural) in these individuals. Study speculates that the CFHR-1-/- deletion is associated with the impaired immune tolerance in these individuals, Gujjar BS, Bhasym A *et al*. 2016 (manuscript in final stage of preparation). Further study also exploring the detail aspects of innate and adaptive mechanisms in these individuals with CFHR-1-/- deletion.

Study is investigating the differences in the cord blood immune phenotypes (particularly monocyte, T cell and B cell subsets) of term appropriate for gestational age (AGAs) with more severely growth restricted small for gestational age (SGAs) neonates, who are known to have adverse outcomes. The initial data show that SGA neonates have comparatively fewer plasmacytoid DCs (pDC), a higher myeloid DC to pDC ratio, more natural killer (NK) cells, and higher serum IgM levels in cord blood. Further, SGA neonates showed a tendency to having relatively more inflammatory monocytes, fewer immature B cells and lower CD4:CD8 T cell ratios. Plan is to follow the above observations in detail. Study also plan to follow the SGA neonates through early infancy to examine the correlations between cord blood immune phenotypes and susceptibility to infection-related morbidity. To understand better the development of the immune systems in neonates, we will analyze the above data of cord blood immune phenotypes of SGA and AGA neonates in correlation with the immune signatures of the adults.

Young Investigator
Masum Saini

Ph. D. Students
Megha Agarwal
Pankaj Kumar
Akashi Sharma
Shreyasi Das
Anushree

Project Assistant
Aakanksha Verma

Project JRF
Shlok Jindal

Sam J. Mathew
Principal Investigator

Intrinsic Signals that Regulate Skeletal Muscle Structure and Function

The research program is aimed at studying the process of cellular differentiation and how it is regulated, using the skeletal muscle as model tissue. In order to do this, investigations are carried out on skeletal muscle development, differentiation, stem cell-mediated regeneration and signaling events underlying tumors which exhibit skeletal muscle characteristics, employing *in vitro* and *in vivo* approaches.

The major goal of this study is to understand the molecular basis of skeletal muscle differentiation, and how aberrant differentiation leads to diseases, for which we have the following objectives: 1. Document the expression dynamics of a family of skeletal muscle specific myosin heavy chain (MyHC) genes, critical to muscle structure and function, during mouse embryonic, fetal and perinatal development. 2. Generate conditional targeted mice to study the function of specific MyHCs in vivo during mouse development, and *in vitro* using gene knockdown in myogenic C2C12 cells. 3. Identify genes with dynamic expression characteristics during C2C12 myogenic differentiation, and investigate their functional requirement in myogenesis. 4. Investigate the role of the regulation of Met signaling in rhabdomyosarcoma, a tumor type wherein the tumor cells exhibit characteristics of skeletal muscle cells at various stages of differentiation.

Myosins are motor proteins vital to cellular processes such as cell motility, cell division and transport of cargo within cells. Among the different classes of myosins, one of the most important ones are the Class II myosins which comprise myosins critical for skeletal muscle contraction. Skeletal muscle contractile myosins are heterohexamers, comprising a pair of Myosin Heavy Chains (MyHCs), a pair of Myosin Essential Light Chains and a pair of Myosin Regulatory Light Chains. We are mainly interested in the MyHCs, to understand their specific roles in skeletal muscle development, differentiation, regeneration and disease.

There are multiple MyHC isoforms in mammals, with most expressed during adult stages, where they form part of the adult skeletal muscle contractile apparatus. Different adult MyHC isoforms exhibit unique contractile properties and their expression in specific muscles is dependent upon functional demand; for instance postural muscles which are frequently used for long durations generally have higher expression of slower MyHCs, which contract slower, utilize oxidative metabolism and are relatively fatigue resistant. In addition to the adult MyHC isoforms, three MyHC isoforms, namely MyHC-embryonic, -perinatal and -slow, collectively known as developmental MyHCs, are expressed by

differentiating muscle cells during embryonic development. Of these, two, namely MyHC-embryonic and -perinatal are exclusively expressed during embryonic stages, while MyHC-slow is expressed during embryonic development and adult life. MyHC-embryonic and -perinatal are re-expressed during adult life in the event of skeletal muscle injury or disease and subsequent stem cell -mediated regeneration. Mutations have been identified in all of these MyHCs that lead to congenital diseases such as myopathies and contracture syndromes like Freeman-Sheldon syndrome. Therefore, it is important to characterize the precise expression dynamics, regulation and function of these developmental MyHCs.

To study the expression dynamics of MyHCs during development, a stage specific analysis of MyHC transcript and protein levels was carried out using quantitative PCR (qPCR) and immunofluorescence respectively on wild type (C57Bl/6) mouse embryos. At the transcript level, MyHC-embryonic and -perinatal had single peaks of expression at E15.5 and E17.5 respectively, whereas MyHC-slow transcripts were expressed at relatively low levels throughout embryonic development. At the protein level, it was found that MyHC-embryonic and -slow were the first MyHCs to be expressed during development, by E10.5, followed by MyHC-perinatal at about E12.5. Interestingly, the protein levels of both MyHC-embryonic and -perinatal underwent 2 peaks of expression at around E14.5 and E16.5 respectively, whereas MyHC-slow protein expression had a single peak at E16.5. These peaks correspond roughly to the 2 phases of myogenesis during embryonic development: the embryonic phase of myogenesis (E10.5-14.5), and the fetal phase of myogenesis (E14.5-17.5), suggesting that these proteins play crucial roles during these myogenic phases. Since the transcript and protein expression did not precisely match with each other, post-transcriptional modifications might be important in regulating the levels of MyHC-embryonic, -perinatal and -slow during vertebrate embryonic development.

The expression of MyHCs during in vitro myogenesis was also characterized using C2C12 mouse myogenic cells as model system, over the course of differentiation. It was found that MyHC-embryonic was the first MyHC to be expressed, followed by MyHC-slow and -perinatal, at the transcript level.

In order to determine the role of MyHC-embryonic in myogenic differentiation *in vitro*, the effect of siRNA mediated MyHC-embryonic knockdown on C2C12 cell myogenic differentiation was also investigated. It was found that MyHC-

embryonic specific siRNA treatment reduced MyHC-embryonic transcript and protein levels significantly. Further, MyHC-embryonic specific siRNA treatment resulted in upregulation of MyHC-slow, initial upregulation of MyoD and Myogenin transcription factors followed by their down regulation at the protein level. This suggests that MyHC-embryonic is normally required to regulate the rate at which myogenic differentiation occurs. In the absence of MyHC-embryonic, the undifferentiated stem-like cells differentiated rapidly, as shown by the upregulation of MyoD and Myogenin differentiation markers. As further evidence for this, we also observed a decrease in the number of reserve cells- the myoblast cells that differentiate to produce myotubes and myofibers, upon MyHC-embryonic siRNA treatment. A similar effect was observed *in vivo* during embryonic and fetal stages of myogenesis.

Another program involves investigations on the expression dynamics of the Transducin-Like Enhancer of Split (TLE) family of core pressors during C2C12 myogenesis, since the *Drosophila* ortholog of the TLEs, *groucho*, has ben suggested to play important regulatory roles in myogenesis. Preliminary results indicate that all 4 TLE genes are expressed during C2C12 myogenesis to varying degrees, and at various stages of differentiation.

Finally, the signals underlying the regulation of the *c-MET* proto-oncogene in a tumor type called rhabdomyosarcoma (RMS) are also being investigated. RMS tumor cells exhibit characteristics of differentiated skeletal muscle cells and although some of the genetic lesions associated with this tumor have been identified, the dysregulation of Met signaling has not been clearly understood. RMS cell lines derived from patients will be used to investigate how Met signaling is regulated in this cancer.

Young Investigator
Sreeshail Sonyal

Ph. D. Students
Hitika Gulabani
Ingole Kishore Dnyaneshwar
Krishnendu Goswami
Mritunjay Kasera
Shraddha Dahale

DBT Research Associate
Jewel Jameeta Noor

Saikat Bhattacharjee
Principal Investigator

Effector-triggered Immunity in Plant-pathogen Interactions: elucidating Inositol-regulated Pathways in Assemblies and Signaling by Immune Regulators

The research programme is investigating molecular processes of effector-triggered immunity (ETI) in plant-pathogen interactions. ETI is induced upon sensing a pathogen effector and involves intricately cross-linked signal transductions that cause massive transcriptional reprogramming towards defense. However, the immune modulators that mediate and messengers that transduce these signals remain unidentified. An *Arabidopsis thaliana-Pseudomonas syringae* pathosystem provides excellent model system for tracing immune signaling routes.

Conventional linear arrangement of transducers and receivers that characterize most signaling cascades are not followed during ETI. Instead, resistance proteins (R) and immune modulators upon activation directly couple with signaling messengers to cause defense-related transcriptome changes. Through preliminary assays, the role of inositol derivatives as key signaling mediators of ETI is suggested. Polar inositol phosphates (InsPs) modulate chromatin organization, transcriptional regulation, mRNA export, hormone signaling etc. Pathogen invasion activates specific phospholipases (PLCs) that generate InsPs from non-polar phosphatidylinositols (PtdIns), present on membranes. This goal of this study is to investigate defense-related roles of InsPs and associated-PtdIns using multi-pronged approaches. Firstly, immune signaling by InsP will be explored and their relative InsP levels in plant mutants with altered defenses determined. Secondly, the study aims to characterize previously identified steady-state protein-protein interactions among immune regulators on lipid interfaces and subsequent processes that investigate roles of pathogen effectors in modulating signaling. Thirdly, inositol-dependent synergistic and antagonistic cross-talk to hormonal pathways will be targeted to understand how pathogen effectors or induced ETI impinge upon the signaling networks.

InsPs are versatile signaling messengers recruited to transduce responses against both biotic and abiotic stresses. Because of the variations in number/position of phosphates attached (Fig. 4) to the central inositol moiety, InsPs introduce multiple levels of complexity in their signaling functions. In cellular processes these variations introduce synergistic or antagonistic functions among the different InsPs.

The phytohormone jasmonic acid (JA) signaling has been demonstrated to be influenced by relative ratio of InsP5/InsP6 in the cell. InsP5 increase leads to degradation of JA-repressors whereas higher InsP6 levels maintains their

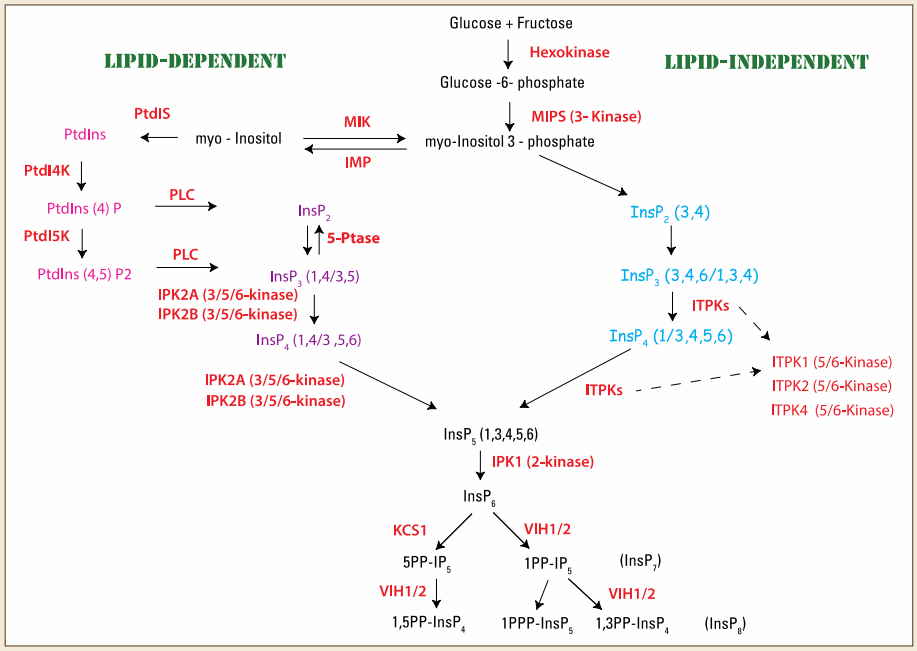


Fig. 4. Schematic representation of inositol biosynthesis and metabolism pathways in plants. Lipid-dependent and lipid-independent synthesis of inositol phosphates (InsPs) and phosphatidylinositols (PtdIns) are shown.

stability there by suppressing JA responses. In defense signaling several inositol biosynthesis/metabolism mutants have been reported to respond differentially to pathogens. The Arabidopsis MIPS1 (a type member of the myo-INOSITOL PHOSPHATE SYNTHASE family). Plants harboring homozygous mutation in MIPS1 (mips1-2) display spontaneous cell death, reminiscent of hypersensitive response that occasionally associate with ETI. Positive defense modulators such as EDS1 and defense hormones such as salicylic acid (SA) are upregulated in mips1-2 plants suggesting that MIPS1 is a negative regulator of defense. However, till date direct evidences indicating the role of InsPs as signaling mediators of immunity is plants remains undeciphered.

To gaining better understanding, the study has undertaken a comprehensive investigation into the roles of specific InsPs in defense responses. Considering the transient nature of an immune trigger, and associated dilutions of responses when analyzing heterogenous cellular populations, the *srfr1-4*plants that constitutively activate defenses are being explored to unravel InsPs roles in ETI. In *srfr1-4* plants increased expression of EDS1 and R proteins that cause enhanced ETI to avirulent strains of *P. syringae* are noted. At a molecular level SRFR1 forms multimeric associations with EDS1 and R proteins in a 'resistasome' complex thereby preventing mis-primed activation of defenses. Interestingly, altered

relative levels of different InsPs caused by differential expression of specific InsP biosynthesis and metabolism genes are detected in *srfr1-4*. Most notably, *MIPS1* and *MIPS2* levels are down-regulated in *srfr1-4* plants. Thus, genetically SRFR1 functions upstream of MIPS. Considering multiple MIPS (MIPS1-3) are down-regulated in *srfr1-4*, a global decrease in myo-inositol concentration is suggested and will be characterized further.

Gene expression databases identify SRFR1expression is co-regulated with *INOSITOL PENTAKISPHOSPHATE KINASE 1 (IPK1)*, an enzyme involved in the biosynthesis of phytic acid ($InsP_6$). Interestingly, it was noted that a T-DNA tagged mutationin*IPK1* (termed *ipk1-1*) caused slight stunting in the mutant plants. Further on, upon generation of an*ipk1-1srfr1-4* double mutant phenotypically *srfr1-4*effects were found epistatic to *ipk1-1*. Enhanced resistance of *ipk1-1* plants to virulent and avirulent *P. syringae* observed suggested that genetically *IPK1* or $InsP_6$ functions as a negative regulator of defense. Whether lower levels of $InsP_6$ or accumulation of lower InsPs (such as $InsP_5$, $InsP_4$ and $InsP_3$) in *ipk1-1*and *srfr1-4* account for the observed enhanced resistance is being investigated. Alteration in various InsPs ratios impinge on phytohormone responses mediated by SA and jasmonic acid (SA). Whether these pathways are being modulated in *ipk1-1* are being tested.

Increased mRNA accumulation in the nucleus of *ipk1-1* plants indicate mRNA export defects. However, initial observations from the study suggest that transcripts of positive defense regulators such as EDS1 and PR1 are elevated and likely does not constitute nuclear-sequestered mRNAs in *ipk1-1*. Whether mRNAs of negative defense regulators are selectively retained in the nucleus or conversely transcripts of positive defense regulators are preferentially exported in *ipk1-1*are being investigated. In preliminary assays indeed the transcripts of the central defense modulator EDS1 were detected to be more enriched in the cytoplasm of *ipk1-1* plants. To obtain a comprehensive view of selective mRNA export, total RNA populations from nucleus and cytoplasm of wild-type and *ipk1-1* plants are being subjected to comparative RNAseq. The results will provide deeper understanding of hypothesized selective mRNA export in defenses. It is interesting to note that *mos3-1* plants that contain a mutation in a nucleoporin also displays mRNA export defects. However unlike enhanced resistance in *ipk1-1, mos3-1* plants are enhanced susceptible to both virulent and avirulent strains of *P. syringae*. This study has detected down-regulation of EDS1 transcripts in *mos3-1* plants. Cross talks between IPK1 and MOS3-regulated nuclear pore

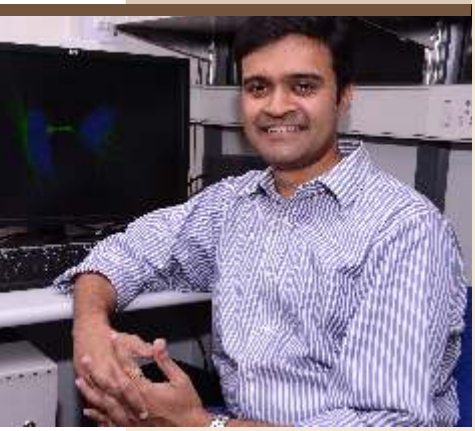
components that may modulate differential export of defense-related transcripts upon immune trigger is being further pursued at a molecular level.

Immune complex assembly is regulated by post-translational modifications (PTMs) and anchors on cytoplasmic membranes via unknown mechanisms. The resident immune regulator EDS1 contains a predicted α/β-hydrolase-like domain present in most phospholipases. Since only microsomal pool of EDS1 associates with resistasome, lipid-dependent anchoring of resistasome via phosphatidylinositols (PtdIns) is suggested. The avirulent effector HopA1 from *syringae* pathovar of P. *syringae* binds specific PtdIns and has been shown to disrupt resistasome associations. To characterize the nature of associations that assemble a resistasome on lipid interfaces,HopA1 functions that perturb this assembly is being characterized. Additionally, the study is also focusing on key immune players such as SRFR1 and EDS1 that contain predicted SUMOylation and SUMO-interaction motifs. Towards this, eds1 containing a lysine to arginine substitution (K478R) in the predicted SUMOylation motif was generated and found to be non-functional in immunity. Interestingly eds1K478R also fails to interact with SRFR1. The results thus strongly suggest SUMOylation-dependent association between EDS1 and SRFR1. In strong support of this, the study detects a global increase in SUMOylation by SUMO1/2 in *srfr1-4* plants. In order to identify the cause of this imbalance qPCR analysis of relative transcript levels of SUMOylation-related genes were performed. Interestingly, increased expression of specific SUMO isoforms and down-regulation of specific SUMO-proteases were detected in srfr1-4. The increased SUMOylation pattern detected in *srfr1-4* plants were noted to be mimicked by exogenous application of SA on wild-type plants. These results strongly suggest that SA elevation is the cause of increased SUMOylated proteins in *srfr1-4*. The molecular mechanisms of SA-dependent changes in SUMOylation and the consequences it has on immune assemblies are being currently undertaken.

Young Investigator
Megha Kumar

Ph. D. Students
Sagar Mahale
Harsh Kumar
Rajaiah Pergu
Amit Sharma
Amrita Kumari
Sunayana Dagar
Chandan Kumar

Research Associate
Pushpa Kumari
(WT-DBT IA Fellow)



Sivaram V. S. Mylavarapu
Principal Investigator

Mechanisms of Cell Division and Cellular Dynamics

The research group is studying the molecular regulation of cellular dynamics. The present focus of scrutiny is to determine the molecular underpinnings of cell division and intercellular communication, two vital and highly dynamic cellular processes.

One major aim of the study is to decipher the molecular mechanisms of mitotic regulation by the Light Intermediate Chain (LIC) subunits of cytoplasmic dynein. Another aim is to uncover the role of the exocytic membrane trafficking machinery during cytokinesis, the physical separation of daughter cells at the end of mitosis. In a separate project, the group aims to elucidate the mechanistic bases for biogenesis and function of novel modes of intercellular communication. The broad overall objective is to obtain a holistic understanding of the molecular mechanisms that govern these processes through a multi-disciplinary approach, involving cell biology, microscopy, biochemistry and proteomics, biophysics and structural biology and model organism development.

LIC2 governs mitotic spindle orientation: The predominant mitotic localization of the LICs at spindle poles suggested that they may contribute to spindle orientation, an important spindle pole-related function of cytoplasmic dynein. HeLa cells treated with LIC-specific siRNAs were visualized by time-lapse imaging on gridded cover slips to identify cells that arrested for prolonged periods (between 80 minutes to 4 hours from nuclear envelope breakdown or NEB) in mitosis to identify LIC-depleted cells. Cells arrested for longer than 4 hours in mitosis often died. It was observed that LIC2 depletion led to drastic spindle mis-orientation as compared to control cells, with over 40% metaphase cells showing a spindle tilt of greater than 20 degrees with respect to the substratum. In contrast, LIC1 depletion showed minimal spindle mis-orientation, suggesting a stronger role for LIC2-dynein. This phenomenon was observed irrespective of the strength of adhesion of the cells to the substratum. LIC2-depleted cells also showed distinctly uneven flattening of daughter cells after anaphase in comparison to control cells that divided parallel to the substratum and thus flattened evenly. In addition, prolonged arrest up to 4 hours by itself did not cause significant spindle mis-orientation. These results together show a major contribution of LIC2-dynein, but not of LIC1-dynein in maintaining proper mitotic spindle orientation.

LIC2-dynein, but not LIC1-dynein transports NuMA asymmetrically to the spindle poles: The next aim was to delineate the molecular mechanism(s) by which LIC2 regulates spindle orientation. NuMA (Nuclear Mitotic Apparatus), a large conserved nuclear protein has been clearly implicated in focusing microtubule minus ends at spindle poles. Both NuMA and dynein are also key components of a crucial cortical protein complex responsible for capturing the plus ends of astral microtubules, thus anchoring spindle poles to the cortex, thereby achieving spindle orientation.

The prominent localization of LIC2 to mitotic spindle poles mirrored the polar localization of NuMA. It was therefore probed whether LIC2-dynein preferentially influenced intracellular NuMA localization. Indeed, the organization of NuMA into well-defined, ring-like structures around mitotic centrosomes in control cells could distinctly be observed. Upon LIC2 depletion, the "upper" poles of (mis- oriented) cells had less NuMA intensity as compared to the lower poles, which was quantified using multiple methods. When all centrosomes were analyzed, depletion of LIC2 did not appear to significantly affect the amounts of polar NuMA immunofluorescence with respect to control cells. However, careful analysis revealed that there was a small but significant reduction (by 15 - 20%) of NuMA intensity preferentially at the "upper" spindle pole upon depleting LIC2 but not LIC1. This result indicated that LIC2-dynein is responsible for mitotic transport of NuMA preferentially to one spindle pole. Spindle pole focusing defects were also observed upon LIC2 depletion, which can partially be attributed to the defective transport of NuMA to the pole, in addition to the role of LICs in centriole cohesion as recently reported by the Allan group. The strong spindle orientation defects observed upon LIC2 depletion prompted an examination of whether there was any change in NuMA localization at the corresponding polar cortex, since cortical NuMA is crucial for ensuring proper spindle orientation. Analysis of fluorescence intensities revealed a significant accumulation of NuMA at the upper cortex only (corresponding to the upper pole) upon depletion of LIC2 but not of LIC1. The fold NuMA accumulation at the upper cortex upon LIC2 depletion tallied with the fold reduction of NuMA at the upper pole. Thus, it was concluded that NuMA is transported preferentially to one spindle pole from the cortex and that this transport is mediated primarily by LIC2-dynein, but not by LIC1-dynein.

LIC2-dynein exclusively interacts with key protein complexes that govern spindle orientation: Cortical NuMA serves as a major recruiting factor for cortical dynein. It was next examined whether LIC1- or LIC2-dynein levels were predominant at

the cortex. Using a multifunctional GFP-tagged IC74 Hela cell line (mfGFP-IC74 Hela), the dynamic localization of mitotic dynein on the mitotic spindle, the spindle poles, the kinetochores and at the pole-proximal cortex could be visualized. Quantification of mfGFP-IC74 fluorescence intensity at the cortex from stills of these movies using published methods showed that the levels of cortical dynein dropped significantly upon LIC2 depletion. A substantial fraction of LIC1- depleted cells showed normal dynein (mfGFP-IC74) levels at the cortex, while all LIC2 depleted cells analyzed showed severely reduced cortical IC74 levels. Quantitative comparison of fluorescence intensities revealed an approximately 2.5 fold reduction in mfGFP-IC74 levels at the cortex upon LIC2 depletion, but a smaller reduction upon LIC1 depletion. The above results together suggest that LIC2-dynein, but not LIC1-dynein carries NuMA from the cortex to the spindle pole during mitosis.

The other major cortically localized protein complex responsible for maintaining proper spindle orientation is the Par3-aPKC complex. Interestingly, Par3 interacts specifically with LIC2 but not with LIC1 in interphase cells, helping to position the centrosome. Given that strong spindle orientation defects were observed only upon LIC2 depletion, the specific interaction of Par3 with LIC2 was probed during mitosis. Mitotically enriched lysates from cells stably expressing affinity tagged (Streptavidin Binding Protein or SBP- tagged) LIC1 or LIC2 were used to probe for the interaction of Par3. Indeed, observed Par3 (~100 kDa isoform) was observed in the affinity pull-downs of LIC2 but not of LIC1 or control affinity

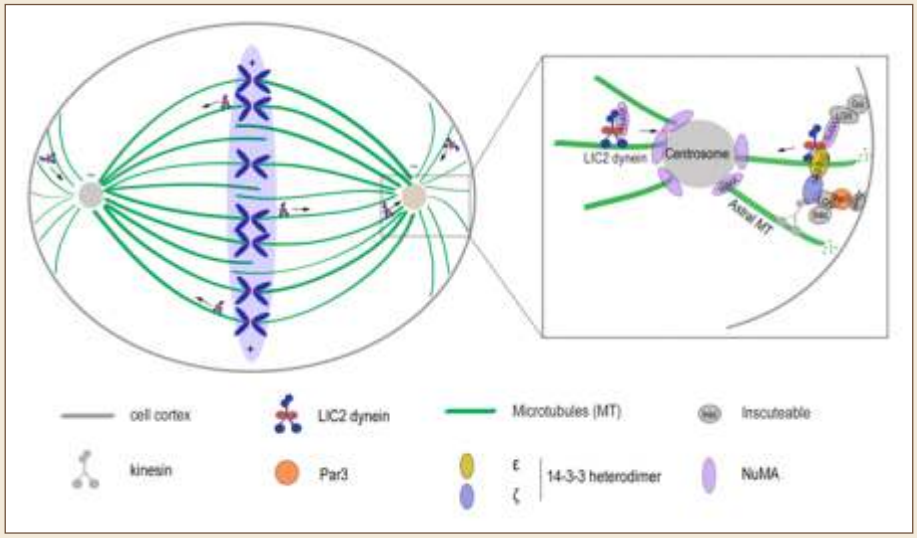


Fig. 5. A model for LIC2-dynein based control of mitotic spindle orientation. LIC2-dynein interacts with NuMA, Par3 and 14-3-3 and anchors astral microtubules to the cortex. LIC2-dynein also transports cortical NuMA along astral microtubules to the spindle poles, thus helping to focus microtubule minus ends at the centrosome. LIC2 dynein may stabilize the bridging of the NuMA- and Par3-mediated spindle orientation pathways through its interaction with the 14-3-3 ε/ζ heterodimer.

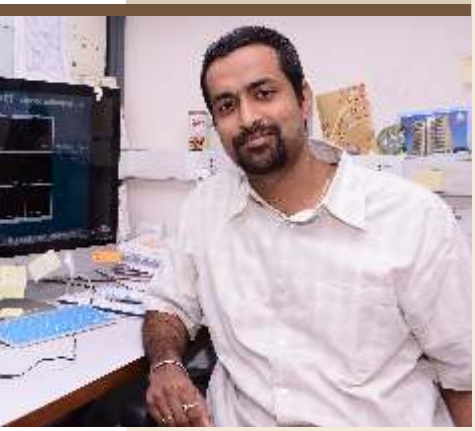
tag alone. This result suggested that the exclusive LIC2-Par3 interaction also exists during mitosis and contributes to the spindle orientation functions of LIC2-dynein. The possible interaction of the LICs with the 14-3-3 proteins E and Z was also probed for in the affinity purification eluates, since these proteins are known to interact with cytoplasmic dynein and to biochemically link the Par3 and NuMA spindle orientation pathways to achieve complete spindle orientation. Robust interaction of both 14-3-3 E and Z proteins with LIC2 was observed, but no interaction was seen either with LIC1 or with the control empty tag alone. Together, the above results revealed an exclusive engagement of LIC2-dynein, but not LIC1-dynein with both the NuMA and Par3 containing cortical protein complexes that collaborate to achieve proper spindle orientation (Fig. 5).

The group has also begun investigating the developmental role(s) played by the LICs during early zebrafish embryogenesis. Depletion of LIC2 by using gene-specific morpholinos led to a distinct, abnormal morphology in embryos. Significantly, all of the morphant embryos die within a few hours, suggesting an essential role for LIC2 in early embryonic development – however embryos treated with sub- optimal does of morpholino remain alive but develop very slowly. Cytological analysis of the morphant embryos at the blastula stage using high-resolution microscopy revealed cell proliferation defects, elongated spindles and dispersed spindle poles in metaphase cells, mirroring the defects seen in mammalian cells. This strongly suggested that LIC2-mediated cell division mechanisms play a vital role in shaping early zebrafish embryo development.

Young Investigator
Prabhakar M

Ph. D. Students
Gayatree Mohapatra
Salman Mustafa Ahmed
Sarika Rana
Pharvendra Kumar
Aamir Sohail
Hridaya Chandrashekhar
Sonalika Maurya
Preksha Gaur

DBT-IA Post Doc. fellow
Mukesh Singh



Chittur V. Srikanth
Principal Investigator

Studies on Biology of Infectious and Idiopathic Inflammation of the Gut

This research programme is focused on understanding the molecular mechanisms that shape infection, inflammation and autoimmune disorders of the gut using a model intracellular gastric pathogen, *Salmonella enterica*var Typhimurium (here after *S. Typhimurium* or ST).

Identification of novel bacterial virulence proteins and their targets within the host that trigger inflammatory signalling are the key objectives of the programme. Furthermore testing if such pathways are operational in states of human autoimmune disorders are the sub-objectives of the programme. The specific areas of interest are described in two sections below:

Section A:Salmonella-host interactions

Gut pathogen *Salmonella Typhimurium* perturbs host SUMOylation machinery: Among the various microbial threats that pose a challenge to the host health, a frequent casual agent of food borne illnesses is *Salmonella Typhimurium* (ST). The disease is called gastroenteritis that results in massive neutrophil infiltration at the site of infection. Remarkably, this is a phenotype also seen in several forms of autoimmune disorders such as Crohn's disease (CD) and ulcerative colitis (UC). Several molecular markers of acute inflammatory state (such as presence of neutrophil-chemotractant hepoxilin-A3, upregulation of multidrug resistant proteins) are shared between these diseases. The study involved investigations of pathways of the gastrointestinal inflammatory conditions using model pathogen ST. Specifically the experiments revealed that host SUMOylation, a post translational modification pathway, was significantly altered by ST during infection. The SUMO machinery, similar to the ubiquitylation machinery, is known to utilize three enzymes that include an activating enzyme E1, a conjugating enzyme E2 (also called as Ubc9) and several ligating enzymes called E3. In a stepwise manner these enzymes conjugate one of three the SUMO substrates (SUMO1 SUMO2 or SUMO3) to lysine residue of target proteins that have a SUMO motif. The SUMO modification could essentially alter several features of the target protein including its localization, function and its ability to interact with other proteins.

Furthermore, the data revealed that ST significantly altered the host SUMOylation in a dynamic manner over the course of infection. Moreover, one of the host targets utilized by ST to modulate the host SUMO machinery was the E2enzyme Ubc9. It was observed that during ST infection Ubc9 protein levels of

were significantly depleted, both *in vitro* and *in vivo*. Multiple approaches were used to pinpoint that the decrease of Ubc9 was due to the presence of a miRNA (miR30c and miR30e). These miRNAs were necessary and sufficient for the *S. Typhimurium* mediated down regulation of host SUMOylation. This was published in MCB in 2015 (Verma et al 2015). To understand the role of SUMOylaton in greater detail, details of intracellular life of ST and its connection to host SUMOylation was investigated. Interestingly data revealed that expression of several members of the host endocytic pathway are modulated by ST in a SUMOylation dependent manner. It was observed that the stability of Rab7, a late endocytic marker depended on the SUMO-status of the cell. Experimental perturbation of SUMOylation machinery resulted in drastic reduction in the protein levels of Rab7, a feature that was reversed by proteasome inhibitor MG132 treatment. This indicated that SUMOylation mediated Rab7 degradation occurred by the action of proteasomal machinery. The mechanistic details of ST infection specific Rab7 stability, function and its connection to SUMOylation would be the future interest of the study.

Section B: SUMOylation machinery is altered in Inflammatory Bowel Disease

To understand the role of SUMOylation in inflammatory bowel disease, Dextran sodium sulphate (DSS) mouse model was used. Groups of mice (6 animals each) were fed DSS mixed in their drinking water for either 3, 4 and 7 days (hereafter referred as DSS-3 mice, DSS-4 mice and DSS-7 mice respectively). These animals were compared with a group that was treated with plain drinking water (referred as control mice). Following the treatment the animals were euthanized and the colonic tissues were isolated for analysis. Among these, DSS-7 mice showed severe signs of inflammation. H&E staining of intestinal cross-sections of proximal colon and ceca of DSS-7 mice, showed more neutrophil infiltrates, loss of crypt, colonic wall thickening, epithelial erosion and edema. In DSS-7 animals, a significant down regulation of E-2 SUMO enzyme Ubc9 ($P<0.05$) was observed relative to control. In spleen there was no decrease in the expression of SUMO genes. The global SUMOylation profiles of DSS-7 mice were altered in the epithelial cell lysates and total tissue lysates of proximal colon and ceca. Immunohistochemistry (IHC) also revealed expression of Ubc9 protein in the epithelial villi near to nucleus in control-mice but a much reduced expression of Ubc9 was observed in the villi of DSS-7 mice.

Down-regulation of SUMO-E2 enzyme and lowered activity of Akt1 in human IBD samples: To investigate if these findings described above are relevant to IBD in humans, the study included experiments in actual human patient samples. Specifically, colonic biopsy samples were obtained by lower endoscopic procedure of patients suffering from UC, CD and control individuals (suspects of IBD who are declared negative for IBD). These experiments were done at the IBD clinic of AIIMS, New Delhi. Samples from these groups of patients were used for gene expression analysis. Sample size for a high degree of statistical power (> 80% &α = 5% per group), based on a pilot study, was calculated to be 22 (n=22) for each group. Hierarchical clustering analysis was carried out using Pearson correlation method. The patients were in the age group 15– 60 years with total N=66 (UC 22, CD 22 and control 22). Compared to control individuals in CD and UC patients the intestines displayed inflammation as evident from qPCR IL-8 mRNA expression analysis. The normalization was done using the housekeeping genes GAPDH and HPRT of control and the disease groups. In case of SUMOylation pathway genes, the expression of Ubc9 displayed drastic repression, between 25-50 fold in some cases and 5-25 fold in other cases was observed. Akin to the mice model, a mild and severe down regulation of Ubc9 was seen among the samples. It was examined if reduced levels of Ubc9 had an effect in global SUMOylation in these tissue samples. SUMO-1 immunoblotting was done in case of the diseased and control patient samples. As can be seen in, a significant down modulation of the overall SUMO conjugation in the UC and CD samples.

To further understand the role of Ubc9 in the pathophysiology of IBD, Study sub grouped the patients with varying levels of Ubc9, such that Ubc9 mildly down regulated samples (between 5-25) of CD were referred to as $CD^{UBC9\text{-}Low}$ and UC as $UC^{UBC9\text{-}Low}$. While the more severely down regulated Ubc9 (between 25-50) in CD were called as $CD^{UBC9\text{-}Hyperlow}$ and UC as $UC^{UBC9\text{-}Hyperlow}$. Interestingly the $CD^{UBC9\text{-}Hyperlow}$ and $UC^{UBC9\text{-}Hyperlow}$ samples displayed a higher mRNA expression of IL8 compared to $CD^{UBC9\text{-}Low}$ and $UC^{UBC9\text{-}Low}$ respectively. Levels of inflammatory and anti-inflammatory cytokines were examined in these samples. Biopsies were taken from 5 patient samples and pooled in each category (as explained in methods) and Enzyme linked Immunosorbent assays (ELISAs) of pro-inflammatory cytokines such as IL6, TNFα and IFNγ was performed. Each of these marker cytokines was seen to be elevated in the CD and UC patient samples. Intriguingly their levels were further elevated in $CD^{UBC9\text{-}Hyperlow}$ and $UC^{UBC9\text{-}Hyperlow}$. Levels of anti-inflammatory cytokines including TSLP, IL10 and TGFβ were seen to be lesser in the $CD^{UBC9\text{-}Hyperlow}$ and $UC^{UBC9\text{-}Hyperlow}$ compared to $CD^{UBC9\text{-}Low}$ and $UC^{UBC9\text{-}Low}$. TGFβ was seen to be slightly higher in the CD and UC samples with no major difference between groups with varying Ubc9.

Immunoblotting of inflammatory markers like pRelA, cJun, and pIκB also showed dysregulated expression in both CD and UC samples. SUMO-modification of all the three molecules is reported in literature. The details of the UC and CD patient clinical parameters revealed correlation of expression of UBC-9 with disease activity index in case of both UC and CD. Such that the UCDAI and CDAI were maximal in case of $UC^{UBC9\text{-}hyperlow}$ and $CD^{UBC9\text{-}hyperlow}$ among UC and CD patients respectively. In case of UC patients, $Ubc9^{HyperLow}$ has more number of patients in relapse as compared to $Ubc9^{Low}$. The status of Akt1 was examined in UC and CD of pooled biopsy samples. As expected these samples also displayed the same pattern of lowering of Akt1 at RNA levels in UC and CD patients samples. Repression was also seen in case of p-Akt1 and akin to the DSS mice model SUMO-conjugated pools of Akt1 were lower in CD and UC as seen by SUMO-1 immunoprecipitation of the protein lysates. The SUMO-conjugated form of Akt1 was further lowered in CDUBC9-Hyperlow samples. The status of phospho-GSK3β was also seen to be lowered in the $UC^{UBC9\text{-}hyperlow}$ patients compared to controls. Together these data revealed that in IBD patient samples, SUMOylation machinery operated at sub optimal levels and this was accompanied with lowered Akt1 activity and profound inflammation. To examine if Ubc9 was capable of governing Akt1 expression and inflammation epithelial cell lines were investigated. In the Ubc9 knocked-down HCT-8 cells, a significant lowering of total Akt1 and pAkt1 was observed. Interestingly Ubc9 upregulation resulted in an increase in the Akt1 protein and pAkt1 levels thus hinting towards a Ubc9 dependent regulation of Akt1.

Overall the study revealed that Ubc9 regulated the expression and stability of Akt1, which in turn was detrimental to functioning of inflammatory regulators and the disease severity of IBD.

Collaborators
Nihar Ranjan Jana, (NBRC)
Neel Sarovar Bhavesh, (ICGEB), New Delhi

Young Investigator
Bharat Singh

Ph. D. Students
Pranita Hanpude
Roshan Kumar
Tanu Johari
Sanjay Kumar
Raniki Kumari
Manisha Kumari
Sandhini Saha

Research Associate
Amit Kumar Dey
Bhoj Kumar

Project Assistants
Tuhin Subhra Haldar
Neha Sharma
Deepali Chhoker
Abhishek Kumar Singh

Tushar Kanti Maiti
Principal Investigator

Post-translational Protein Modifications: Involvement in Cellular Processes and Disease Regulation

The research group is studying how post-translational modifications of protein regulate diverse cellular signaling events and their mechanism of disease regulation.

Protein posttranslational modifications (PTM) such as phosphorylation, ubiquitination, SUMOylation, redox-modification, acetylation and glycosylation play an important role in different cellular processes including protein quality control, cell cycle regulation, endocytosis, DNA repair, vesicles trafficking and so on. Dysregulation of these processes lead to different diseases like cancer and neurodegeneration. One of the important aspects of this research program is to understand the ubiquitin signaling mechanism and their regulation in cellular pathway and diseases. Human genome analysis and proteomics data reveal almost one hundred deubiquitinating enzymes, which majorly regulate the ubiquitin homeostasis in cells. However, the molecular functions of most of the DUBs are still elusive. Study is investing the molecular basis of their involvement in cellular functions like protein degradation, histone modification, and endocytosis of plasma membrane proteins. It has also been revealed that the dysregulation of deubiquitinating enzymes leads to diseases like Parkinson, Alzheimer, ataxia, heart disease and different types of cancer. Study aims are to understand the possible molecular mechanism underlying these diseases. Another aspect of this research program is to understand how redox modification particularly Cys-nitrosylation regulates diverse cellular processes including disease outcome from neurodegenerative disease to microbial infection.

Molecular basis of BRCA-1 Associated Protein-1 (BAP1) function in cellular processes and disease regulation: Since last three decades considerable progress has been made in genome sequencing field that reveals genomic landscape of cancer. Advancement of genomic studies has shown that there are more than hundred genes altered due to intragenic mutation. These mutations are essential for oncogenic progression. In a specific tumor type there are some driver genes that regulate core cellular processes like cell fate, cell survival and genome integrity. BRCA1 associated protein 1 (BAP1) is a nuclear deubiquitinase that regulates tumor suppressor activity and widely involves many cellular processes ranging from cell cycle regulation to gluconeogenesis. Impairment of enzymatic activity and nuclear localization induce abnormal cell proliferation. It

is considered to be an important driver gene, which undergoes frequent mutations in several types of cancer. However the role of mutation and oncogenic gain of function of BAP1 are poorly understood. The cellular localization, enzymatic activity and structural changes for four missense mutants of the catalytic domain of BAP1, which are prevalent in different types of cancer have been investigated. These mutations have triggered cytoplasmic/perinuclear accumulation in BAP1 deficient cells, which is observed in proteins that undergo aggregation in cellular condition. Amyloidogenic activity of mutant BAP1 has been revealed from its reactivity towards anti oligomeric antibody in HEK293T cells. The structural destabilization in the catalytic domain mutants, which has eventually produced beta amyloid structure is characterized by atomic force microscopy study. The cancer associated mutant up-regulates heat shock response and activates transcription of genes normally co-repressed by BAP1. The study unambiguously demonstrates that structural destabilization and subsequent aggregation abrogate BAP1's cellular mechanism leading to adverse outcome.

The crystal structure of BAP1 is still not solved and the homology modeling study has shown similar catalytic domain architecture like UCHL5. Recent enzymatic investigation has confirmed that the enzymatic parameters like Km, kcat and kcat/Km are very much similar to UCHL5 which provides a strong evidence of common ancestry of BAP1 and UCHL5 regulators. As BAP1-ubiquitin binding structure is not known, the study has undertaken a biochemical approach to understand how this enzyme recognizes the substrate using combined Isothermal Titration Calorimetry (ITC), Surface Plasmon Resonance (SPR) and enzymatic kinetics studies. The ubiquitin binding experiment and thermodynamics analysis of UCH family members have been carried out. The ITC thermodynamics data have shown that UCHL3 has enthalpically favourable interaction which indicates a strong hydrogen bonding and ionic interaction. The ubiquitin interaction with UCHL1 and UCHL5 is contributed by hydrogen bonding, ionic interaction as well as strong hydrophobic interactions (increase in DS). However, BAP1 shows entropically favourable interaction where not only hydrophobic interactions but also change of protein conformations are involved. The association constant of ubiquitin with BAP1 is commensurate with Km and SPR binding data.

S-Nitrosylation of UCHL1 induces structural instability and promotes A-synuclein aggregation: *A new mechanism in Parkinson's disease pathology:* Parkinson's disease (PD), one of the most common neurodegenerative movement disorders, is known to cause abnormal motor neuron functions like rigidity, resting tremor and postural instability. It is characterized by a progressive loss of dopaminergic neurons in midbrain particularly substantia nigra. The majority of PD cases reported are sporadic. However, almost 10% PD cases are familial in nature. Several genes have been identified to cause a familial PD and among them α-synuclein, PINK1, parkin, DJ-1 and LRRK2 have been well

studied. The clinical as well as experimental observations support the hypothesis that increased expression of α-synuclein is important for PD pathogenesis. The increased cytoplasmic expression of α-synuclein in aged human brain is one of the major risk factors for PD development. Genome wide association studies reveal that single nucleotide polymorphisms associated with α-synuclein are linked to the increase risk of PD. Alpha-synuclein is a major component of cytoplasmic inclusions called Lewy body (LB) in sporadic PD patient brain which indicates that α-synuclein plays a decisive role in the pathogenesis of PD. However the mechanism underlying the formation of LB remains poorly understood. Mass spectrometry analysis of LB has identified almost 40 proteins of different families. The question remains unclear how other LB proteins regulate or influence the α-synuclein aggregation that leads to loss of neuronal function.

Ubiquitin C-terminal Hydrolase-1(UCHL1) is a neuron specific deubiquitinating enzyme, which constitutes up to 2% of the soluble brain protein and plays a key role in Parkinson's disease (PD). It is one of the most important proteins, which constitute Lewy body in PD patient. However, how this well folded highly soluble protein is present in this proteinaceous aggregate is still unclear. Study reports here that UCHL1 undergoes S-nitrosylation *in vitro* condition, cell culture model and rotenone induced PD mouse model. The preferential nitrosylation in Cys 90, Cys152 and Cys220 has been observed which alters the catalytic activity and structural stability. Study shows here that nitrosylation induces structural instability and produces amorphous aggregate, which provides a nucleation to the native α-synuclein for faster aggregation. The findings provide a new link between UCHL1-nitrosylation and Parkinson's disease pathology.

Structural Biology of Host-Microbial Interactions in Health and Diseases

Young Investigator
Shivendra Singh

Ph. D. Students
Priyanka Chaurasia
Abhiruchi Kant
Abhin Kumar Megta
Rajnesh Kumari Yadav
Jyotsna Misra

Research Associate
Arjun Kumar Mishra
Tapas Bhattacharyya

Vengadesan Krishnan
Principal Investigator

Objective of the research programme is to explore and understand the structural basis of host-microbial interactions in health and disease. Presently, the study focuses on cell surface proteinaceous assemblies like pili from beneficial and pathogenic bacteria, and aims to elucidate common and specific bacterial strategies in probiosis and pathogenesis.

Adherence to host surfaces is a key step in bacterial colonization regardless of whether it harms or benefits the host. This first level of host-bacterial interaction is regarded as prerequisite for bacterial colonization. All the later events highly depend on the success of adherence. During the adhesion process, bacteria have to go through several challenges including host immunological and physical clearances. To avoid being removed from the host surfaces, bacteria often assemble hair-like appendages known as pili or fimbria on their cell surfaces. These surface organelles enable bacteria to effectively and quickly initiate adherence to the host. The pili have also been implicated in other processes such as conjugation, twitching motility, immunomodulation, biofilm formation, etc. Different types of pili are known in pathogenic bacteria and they have been considered as virulence factor due to their significant contribution to adherence and virulence. Because of their immunogenic properties and exposure on the cell surface, they have also been proposed as ideal vaccine candidates in preventing bacterial infections. There is a considerable growing interest in understanding the basis of pili-mediated interaction.

Targeting pili-mediated bacteria-host interface is seen as a promising approach in infection control, disease treatment and health improvement. The structural biology has been instrumental over the last few years in obtaining structural insights of pilus biogenesis, adherence and interaction in pathogenic bacteria. However, the pili in commensal/probiotic bacteria are relatively recent and not much is known. The structural investigation program on pilus constituents in beneficial bacteria has been initiated towards understanding their unique structural features that may distinguish from their counter parts in pathogenic bacteria. These features may lie in pilus biogenesis, architecture, cellular targets, and mode of adhesion/interaction. This appears to help them to reduce their degree of virulence, to compete for adhesion site and act as colonization barriers for pathogens attachment, to survive and persist within the host without harming them, and to stimulate immune system and provide health benefits. Structural investigations have begun with pilus constituents in *Lactobacillus rhamnosus* GG, which is one of the well-known and widely used probiotic strains. *L. rhamnosus* GG genome contains two pilus gene clusters named *SpaCBA* and *SpaFED*. The *SpaCBA* encodes a major pilin SpaA, two ancillary pilins SpaB and SpaC, and a pilin-specific sortase SrtC1. Similarly, the *SpaFED* operon contains genes for a major pilin SpaD, two ancillary pilins SpaE and SpaF, and a pilin-specific sortase SrtC2.

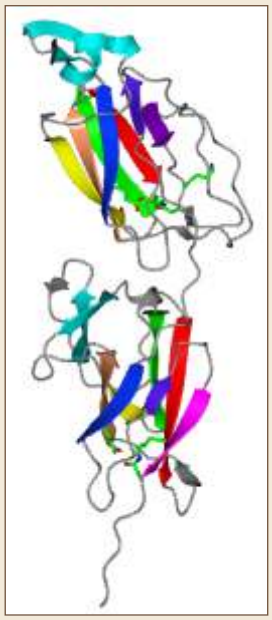


Fig. 6. Structure of SpaA.

Fig. 7. Pilus shaft of SpaA.

The crystal structure of major pilin SpaA has been obtained by fragmented approach. This is the first crystal structure of a pilin subunit from a non-pathogen host. SpaA is made up of two tandem CnaB domains, which are variants of immunoglobulin (Ig) fold (Fig. 6). Each domain harbors an isopeptide bond with a canonical E-box motif. Interestingly, the isopeptide bond in the C-terminal domain forms between lysine and aspartate while the one in the N-terminal domain forms between lysine and asparagine as seen in several structures of backbone pilins from pathogens. Though the folds of individual domains are similar to their counterparts in pathogenic strains, there are differences in the loop regions, isopeptide bond formation, position of linking lysine, and domain orientations. Several mutants were made to study the role of glutamate and isopeptide bond in thermal and proteolytic stability, isopeptide bond-mediated conformational changes, and pilus polymerization. The proteolytic assay and circular dichroism spectroscopy experiments suggest that the glutamate residue in the E-box affects the proteolytic and thermal stability of SpaA. However, a cumulative effect perturbing normal pilus polymerization was unobserved. Crystal structure of the mutant SpaA proteins were obtained and analyzed. The glutamate substitution in the N-domain (E139A) prevents isopeptide bond formation in the N-domain and maintains the overall fold with some minor changes. Whereas, a similar substitution in the C-domain (E269A) causes different conformational effects over time. Initially E269A has a tendency to destabilize protein folding when isopeptide bonds are absent. However, later surprisingly, it retains the overall fold following the formation of intact isopeptide bonds. Crystal structure of truncated N-domain was obtained from E269A mutant protein, and it shows how the movement of adjacent domains or the C-terminal end can affect isopeptide bond formation and conformation. In pathogen hosts, the N-domain is known to be labile and has never been previously elucidated by X-ray crystallography. Notably, substitution of aspartate to asparagine as in pathogens host (D295N) does not affect isopeptide bond formation in the C-terminal domain irrespective of E269 being present or absent.

Intriguingly, the three molecules present in the asymmetric unit of SpaA crystal are arranged in a "head-to-tail" arrangement that may reflect its native biological state (Fig. 7). This arrangement places the C-terminal sorting motif of each molecule into a cavity-like space nearby the pilin motif of the adjacent molecule. Along the pilus axis, each SpaA molecules shows a rotation angle of ~120° and translation of ~78 Å. The inter-domain hinge angle (152°) in each molecule and head-to-tail stacking of successive molecules forms an elongated

spiral staircase-like arrangement. This could possibly offer both flexibility and rigidity to SpaCBA pilus for sampling the host surface and facilitating attachment.

SpaD has been crystallized in two forms. Initially it was crystallized in space group $P2_12_12_1$, with unit cell parameters a = 47.79 Å, b = 72.78 Å, c = 400.08 Å and two molecules in asymmetric unit. Later in space group $P6_522$ with unit cell parameters a & b = 73.91 Å, c = 430.01 Å and one molecule in the asymmetric unit. Both crystals forms were diffracted nearly to 2.8 Å at the synchrotron source

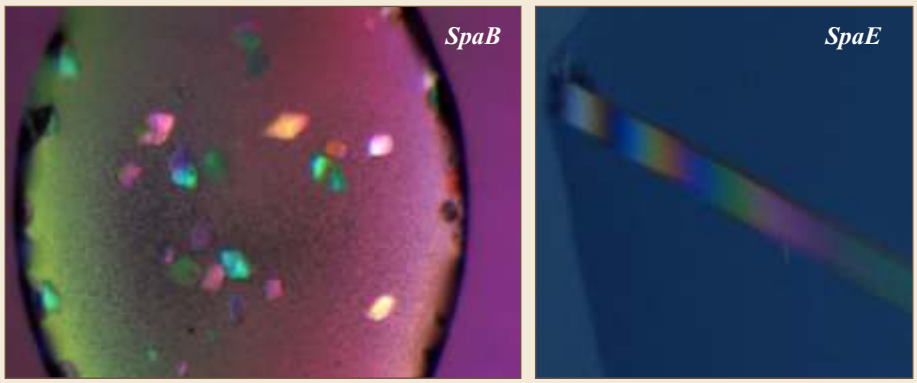


Fig. 8. Crystals of basal pilins.

but with anisotropic and streaky diffraction patterns. Specific experimental setup enabled the spots resolving and data processing. Attempts are underway to solve the structure by MR-SAD using the C-terminal fragments derived by limited proteolysis and halide quick soaking, and selenium derivative data.

SpaC has been successfully crystallized in the presence of magnesium ions. X-ray diffraction data were collected to 2.6 Å resolution. The SpaC crystal belongs to the space group $P2_12_12$ with unit cell parameters a = 116.5 Å, b = 128.3 Å, c = 136.5 Å and contains two molecules in the asymmetric unit. The basal pilins, SpaB and SpaE has been purified in soluble and pure forms. Recently, some crystallization hits were observed (Fig. 8). Crystallization conditions are being optimized to produce diffraction quality crystals. Purification of pilus components from *L. ruminis* and primary colonizers of oral biofilm are being attempted.

Academic Activities

Academic Activities

Multidisciplinary PhD Programme

A multidisciplinary doctoral programme has been instituted for students who have completed Master's in the relevant disciplines of natural sciences, medicine, engineering and related fields of study. Currently, scientific research in RCB is carried out in the areas of cell, chemical, computational, developmental, plant and structural biology, tissue engineering, analysis of complex diseases for identification of intervention points, host-pathogen interactions and development of knowledge-based drug discovery strategies. Candidates who have obtained Master's degree in any field of Science (or equivalent) with an intense interest to work at the interface of multiple disciplines are enrolled as Junior Research Fellows (JRFs). The research fellows are mentored and guided by faculty who are specialized in their areas of research. The fellowship is initially tenable for the duration of two years and is extendable for additional three years after a review. JRFs are recruited once during the academic year. As on the current academic year, 77 students are enrolled for the PhD programme.

Young Investigator (YI) & Post-Doctoral Fellow Programme

YI Awards scheme has been initiated to nurture outstanding recent PhD fellows with the aim to pursue novel discoveries under the mentorship of the RCB Faculty. Under this programme, the Centre enrols young scientists from within India and outside the region to conduct research in different areas of biotechnology. During the past year, the YI programme has been augmented and suitable candidates have been recruited. In addition to YIs, 11 post-doctoral fellows are also being mentored by the RCB faculty funded through various extramural projects.

Short-term Training

Unique short programmes for post-graduate students in the relevant fields of biotechnology are introduced where these students have the opportunity to conduct short research projects and/or dissertation work towards partial fulfilment of their degrees. Over 100 students have benefitted from this programme so far.

In addition, short-term training programmes in platform technologies towards skill development in multiple areas such as cell and tissue engineering, nano-biosciences, biomedical engineering, climate sciences and energy resource management are planned.

Post-graduate Programmes

The RCB Act 2016 has empowered the Centre to grant degree and post-graduate diploma in biotechnology and related subjects at the interface of varied disciplines. The Centre is initially conceptualizing a 2-years MSc program and/or a 6-years integrated MSc-PhD program in biotechnology.

In addition to the above courses, the centre has plans to offer four short courses leading to skill needs of biotechnology industry sector at various levels. These programmes shall be offered in partnership with Industry.

RCB has also taken the lead in designing a unique Master's level degree programme in Medical Sciences. The programme is designed with the objective of providing knowledge in life sciences with an emphasis on human biology, clinical and translational research. The overall aim of this course shall be to build the aspects of practical learning and research skills for medical and related graduates that will enhance their educational experience and contribute to the making of competent medical researchers.

Seminars by visiting scientists

S. No.	Name of Scientists and affiliation	Title/ Topic of the seminar
1.	Dr. Shri Ram Yadav, Indian Institute of Technology, Roorkee, India.	A near-death experience during phloem cell differentiation and symplastic cell-cell communication during Arabidopsis vascular development
2.	Dr. Parul Mishra University of Massachusetts Medical School, Worcester, MA, USA	Mechanistic insights into the *in vivo* function of Heat shock protein 90
3.	Dr. Pinky Kain Sharma University of California, Riverside, USA	Neural circuitry for detection of insect repellent DEET and attractive sugar in Drosophila
4.	Dr. Hemant Kumar Department of Biochemistry and Biophysics, University of California, San Francisco, USA	Major facilitator Super-family: Biophysics to Biochemistry
5.	Prof. Maria Leptin Director European Molecular Biology Organisation (EMBO) and Professor, University of Cologne, Germany	Cell shape and Morphogensis: Subcellular and Supracellular mechanism
6.	Dr. Jose Sebastian, Carnegie Institution for Science, USA.	Grasses suppress shoot-borne roots to conserve water during drought
7.	Dr. Prem Singh Kaushal Wadsworth Center, Albany, USA	Cryo-Electron Microscopy (cryo-EM) studies of ribonucleoprotein complexes: the group II intron and ribosomes
8.	Dr. Sathees C. Raghavan Department of Biochemistry, Indian Institute of Science, Bangalore, India	Repair of DNA double-strand breaks: the good, the bad and the unknown
9.	Dr. Sudip Mondal, University of Texas Austin TX USA	High resolution three dimensional imaging of C *elegans*
10.	Prof. K Subramaniam Indian Institute of Technology-Madras, Chennai, India	A decade-long \obsession with PUF-8 and germ cell decisions
11.	Dr. Madhusoodanan Urulangodi IFOM-The FIRC Institute of Molecular Oncology, Milan, Italy	Replication fork restart mechanisms at the damaged fork.

S. No.	Name of Scientists and affiliation	Title/ Topic of the seminar
12.	Prof. B. J. Rao Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India	Replication fork restart regulation is critical in any proliferative cell: the role of ATR in attenuating its own signaling in mammalian cells
13.	Dr. Jayanta Bhattacharyya Associate in Research Duke University, USA	Genetically engineered polypeptide for drug delivery
14.	Dr. Sambashiva Banala Post doc Associate Howard Hughes Medical Institute, USA	Development of Novel Chemical Tools for biological applications
15.	Prof. Joel L. Sussman, Weizmann Institute, Israel	Recent Breakthroughs in the Structure/Function Studies of Acetylcholinesterases

Workshops

The UNESCO India Cluster Office and RCB organized a "Regional Dialogue on Science and Technology Policy in the context of Biotechnology towards Sustainable Development" from 29-30 Dec, 2015. Eminent speakers, policy makers and academic institutions from more than 6 countries took part in this meeting. The delegates presented and deliberated on the country's S&T policy and the improvements made in the last three decades with special focus on biotechnology. This event was very unique wherein policy makers, academic institutions, professional bodies and industry associations and other such stakeholders presented country specific strategies and recent developments in human health, crop biotechnology and environmental strategies. Overall, the meeting facilitated an important exchange of information and ideas regarding policies associated with the role of biotechnology in sustainable development.

The Centre organized an International Workshop on computational crystallography titled "CCP4 Workshop 2016: Computational Biology at the Nanoscale". The workshop was organized and conducted in partnership with the Collaborative Computational Project No. 4 (CCP4) executive (United Kingdom). The workshop educated young scientists regarding new developments in computational crystallography and provided hands-on training regarding the use of new software. The workshop was attended by 60 young scientists from

across the country and 19 instructors from India and abroad. The workshop ended with a mini-symposium titled "Form & Function in Biology" with lectures from distinguished scientists within India

RCB organized the eighth edition of the Young Investigators Meeting (YIM-2016), a conference for Post-Doctoral fellows invited from international universities across the globe. The workshop serves as a platform for young scientists to interact with mid-career and senior investigators and provides an opportunity for the development of fruitful collaborations between scientists at different levels of their careers.

RCB hosted the 2015 edition of the Ramalingaswami Fellowship Conclave. The conclave was attended by more than 150 Ramalingaswami Fellows from across the country. The sessions of the conclave were mentored by 25 eminent scientists. The conclave is a unique programme of DBT to engage with scientists across the institutions where such fellows gather to share their recent developments, achievements, progress and experiences.

Prof. Joel Sussman conducted a workshop regarding the Proteopedia database titled "Proteopedia- a scientific "Wiki" bridging the rift between 3D structure & function in Biomacromolecules". The workshop educated students from within RCB and other institutes in the National Capital Region about the Proteopedia website which provides details about structure-function relationships regarding different proteins.

RCB and National Institute of Advanced Industrial Science and Technology (AIST), Japan have collaborated in jointly organizing advanced training programmes in biomedical imaging technologies and biotechnology. A joint laboratory called the DBT-AIST International Laboratory for Advanced Bio-imaging (DAILAB) was inaugurated on 10th December 2015 at the NCR Biotech Science Cluster by Dr. Y. Kamagata, Deputy Director General (AIST) at the RCB. The laboratory will serve as a bioimaging platform for scientists working at the interface of biomedical and health related areas. The scientists and leaders who spoke on the occasion were Dr. Subrata Sinha, Executive Director of RCB, Dr. Y. Ohmiya, Director of AIST, Dr. Sunil Kaul and Dr. Renu Wadhwa, Prime Senior Researchers of AIST.

Lectures delivered/ Conferences attended/ Visits abroad

Dr. Avinash Bajaj

1. Presented poster titled 'Engineering of self-assembled phospholipid based bile acid-drug conjugates/nanoparticles for cancer therapy with reduced toxicity in non-human primates: A new revolution in drug delivery' at AACR special conference on Engineering and Physical Sciences in Oncology held at the Westin Boston Waterfront in Boston, USA during June 25-28, 2016.
2. Presented poster titled 'Engineering of biocompatible hydrogels for sequential and sustained release of anticancer drugs for combination cancer therapy' at AACR special conference on Engineering and Physical Sciences in Oncology held at the Westin Boston Waterfront in Boston, USA during June 25-28, 2016.
3. Co-authored poster titled "Sphingolipid Profiling using Robust and Sensitive LC-MD/MS Method" presented at Annual Conference of American Society for Mass Spectrometry held at Antonio, USA during June 5-9, 2016.
4. Attended 12th International Conference of the Asian Clinical Oncology Society held at New Delhi, India during April 8-10, 2016.
5. Delivered an invited lecture titled 'Introduction to Nanotechnology: Future of Every Disease' at Bhaskaracharya College of Applied Sciences, University of Delhi on April 22, 2016.
6. Delivered an invited lecture titled 'Unlocking the chemistry of bile acids in combating cancer and infectious diseases' at Amity University-Haryana, Manesar on 25th April, 2016.
7. Attended Tata Memorial Centre Platinum Jubilee Conference titled "A Conference of New Ideas in Cancer-Challenging Dogmas" held at National Centre for Performing Arts, NCPA Marg, Nariman Point, Mumbai during February 26-28, 2016.
8. Delivered an invited lecture titled 'Emerging Technologies for Cancer Therapy' at the "International Workshop on Cancer Awareness, Prevention, Screening & Early Detection for SAARC Nations" held at Delhi State Cancer Institute, New Delhi, India during 29 February-03 March 2016.

Dr. Deepak T. Nair

1. Delivered an invited lecture "Lessons in crystallography from studies on DNA polymerases" at the 44th National Seminar in Crystallography held at IISER-Pune during July 10-13, 2016.
2. Delivered Plenary lecture titled "Mechanistic insights into role of DNA polymerase IV in replication and evolution illuminate a novel strategy to combat multidrug resistance" at the 16th Conference of the Science Council of Asia held at Colombo, Sri Lanka during May 30-June 1, 2016.
3. Delivered Mentor lecture titled "A close look at an error-prone DNA Polymerase: An evolving story" at the 8th Young Investigator Meeting (YIM-2016) held at Heritage Resort, Manesar during February 27- March 2, 2016.

4. Delivered invited lecture titled "Reactive Oxygen Species, Antibiotics and a DNA polymerase." at the 1st Winter Symposium of the National Institute of Immunology Alumni Association (NIIAA) held at NII, New Delhi during December 23-24, 2016.
5. Organized the workshop titled "Computational Biotechnology at the Nanoscale: CCP4 Workshop 2016" held at Regional Centre for Biotechnology, Faridabad during February 15-20, 2016.
6. Delivered invited lecture "License to Methylate: Mechanism of low-pH induced activation of a Type III DNA methyltransferase from H. pylori" at the conference titled "Bacterial Expressions II" held at NCBS, Bangalore from December 1-5, 2015.

Dr. Sam Mathew

1. Visited the Department of Human Genetics, University of Utah, USA, as part of the "work outside the host institution" scheme of the Welcome Trust/DBT India Alliance Intermediate Fellowship awarded for the project "The role of developmental myosin heavy chains in skeletal muscle development, regeneration, homeostasis and disease" during December 5, 2015- January 6, 2016.
2. Organized the 8th Young Investigator Meeting (YIM-2016) held at Heritage Resort, Manesar during February 27- March 2, 2016.
3. Visited the Department of Human Genetics, University of Utah, USA, as part of the "work outside the host institution" scheme of the Welcome Trust/ DBT India Alliance Intermediate Fellowship awarded for the project "The role of developmental myosin heavy chains in skeletal muscle development, regeneration, homeostasis and disease" during May 29- August 1, 2016.

Dr. Vengadesan Krishnan

1. Delivered an invited talk on 'Structure of pilus protein SpaA provides insights into SpaCBA pilus formation in probiotic Lactobacillus rhamnosus GG' at the 2 International Conference on Structural and Functional Genomics held at SASTRA University, Thanjavur during August 19-20, 2016.
2. Delivered an invited talk on 'Structural biology of Pilins in Gram-positive bacteria' at the 56th Annual conference of Association of Microbiologists of India held at Jawaharlal Nehru University, New Delhi during December 7 - 10, 2015.
3. Presented poster titled 'Successful structure solution for shaft pilin SpaA from Lactobacillus rhamnosus GG using fragmented approach and distant homolog - structural insights into SpaCBA pilus assembly' at the 13th Conference of Asian Crystallographic Association (AsCA) held at Kolkata during December 5 - 8, 2015.
4. Attended The 27th DeLCON Meeting (Nodal Officers Meeting) held at Institute of Life Sciences (ILS), Bhubaneswar during April 25 - 26, 2016.
5. Attended Global Biotechnology Summit held at Vigyan Bhavan, Central Secretariat, New Delhi, India, during February 5 - 6, 2016.
6. Attended "Regional Dialogue on Science & Technology Policy in the context of Biotechnology" held at Regional Centre for Biotechnology, Faridabad India, during December 29 - 30, 2015.
7. Visited BM14 beamline at European Synchrotron Radiation Facility (ESRF), France during July 5-12, 2016.

8. Co-organized the 5th Annual Ramalingaswamy Conclave held at Regional Centre for Biotechnology, Faridabad, India, during December 18 - 20, 2015.
9. Co-organized "Computational Biotechnology at the Nanoscale: CCP4 Workshop" held at Regional Centre for Biotechnology, Faridabad during February 15 - 20, 2016.

Dr. Deepti Jain

1. Delivered an invited lecture titled "ATP induced structural remodeling in the antiactivator FleN enables formation of the functional dimeric form" at the 44th National Seminar on Crystallography July 10-13, 2016 at IISER-Pune.
2. Co-organized "Computational Biotechnology at the Nanoscale: CCP4 Workshop" held at Regional Centre for Biotechnology, Faridabad during February 15 - 20, 2016.

Dr. Saikat Bhattacharjee

1. Attended 3rd International Plant Physiology Congress on "Challenges and Strategies in Plant Biology Research" held at Jawaharlal Nehru University, New Delhi during 11-14 December 2015.
2. Co-organized the 5th Annual Ramalingaswamy Conclave held at Regional Centre for Biotechnology, Faridabad, India, during December 18 - 20, 2015.
3. Delivered an invited seminar titled "Effector-triggered immunity against a phytopathogenic bacterium - Regulation, perturbation and Signaling" at Arabidopsis 2016 Meeting held at IISER, Mohali during March 20-22, 2016.

Dr. Divya Chandran

1. Delivered an invited lecture titled "Engineering durable powdery mildew resistance in food legumes" at the 56th Annual Conference of Association of Microbiologists of India at Jawaharlal Nehru University, New Delhi during 7-10 August 2015.
2. Attended 3rd International Plant Physiology Congress on "Challenges and Strategies in Plant Biology Research" held at Jawaharlal Nehru University, New Delhi during 11-14 December 2015.
3. Attended and presented a poster entitled "Modulation of host immunity and nutrient allocation by a biotrophic pathogen" at the 8th Young Investigators' Meeting held at the Heritage Village Resort, Manesar, Gurgaon during 27 February-3rd March 2016.

Dr. Sivaram Mylavarapu

1. Presented an invited talk titled "Motoring Through Cell Division" at the 1st Winter Symposium of the National Institute of Immunology Alumni Association held at NII, New Delhi from December 23-24, 2015.
2. Attended and presented the Vote of Thanks at the "Regional Dialogue on Science and Technology Policyin the Context of Biotechnology" held at the Regional Centre for Biotechnology Faridabad from December 29-30, 2015.
3. Made a presentation on the genesis and proposed activities of the DAIlab at RCB on the occasion of the inauguration of the DAIlab@RCB at the Regional Centre for Biotechnology, Faridabad on December 10, 2015.

Memberships of Professional/ Academic Bodies/ Editorial Boards

Dr. Prasenjit Guchhait

- Member for the Society for Biological Chemist, India 2013-present
- Member of the Pineal Study Group, India 1999-present
- Member, Editorial Board for the Austin Hematology 2016-present
- Member, Editorial Board for the Cardiology: Open Access 2016-present
- Member, Editorial Board for the Journal of Hypertension and Cardiology 2012-present
- Member, Editorial Board for the World Journal of Hypertension 2010-present

Dr. Deepak T. Nair

- Member, Society of Biological Chemists
- Member, Indian Crystallographic Association
- Member, Guha Research Conference

Dr. Deepti Jain

- Member, Society of Biological Chemists
- Member, Indian Crystallographic Association.

Dr. Saikat Bhattacharjee

- Review Editor, Plant Biotic interactions, Frontiers in Plant Science, Frontiers Publishing Group.

Dr. Divya Chandran

- Review Editor, Frontiers in Plant Science: Plant Biotic Interactions, Frontiers Publishing Group

Dr. Vengadesan Krishnan

- Member, Indian Crystallographic Association
- Member, Indian Biophysical Society
- Member, International Union of Crystallography

Dr. Sivaram Mylavarapu

- Member, Indian Society of Cell Biology
- Member, Society of Biological Chemists

Distinctions, Honours and Awards

Dr. Prasenjit Guchhait

1. Ad-hoc reviewer for research grants of DBT and DST, Govt. of India 2015-present
2. Ad-hoc reviewer for journals: Antioxidants and Redox Signaling; Frontier in Bioscience; Journal of Thrombosis and Thrombolysis; British Journal of Hematology; Haematologica, Plos One; Journal of Immigrant and Minority Health; Scientific Reports.
3. Ad-hoc reviewer for Bold Face Editors, The International Scientific Editorial Service 2012-present

Dr. Deepak T. Nair

1. Recipient of National Bioscience Award for Career Development (2014) from Department of Biotechnology, Government of India.
2. Mentor at the 8th Young Investigator Meeting (YIM-2016)
3. Chair of the Session titled "Protein-Nucleic Acid Interactions" at the 44th National Seminars in Crystallography-44
4. Session chair at the Annual Talks of the National Centre for Biological Sciences (Theme: Coming of Age: Transitions in Biological Systems.)

Dr. Deepti Jain

1. Recipient of SERB Young Investigator Award, Department of Science and Technology, Government of India
2. Recipient of Innovative Young Biotechnologist Award, Department of Biotechnology, Government of India
3. Session Chair at the 1st Winter Symposium of the National Institute of Immunology Alumni Association (NIIAA) held at NII, New Delhi during December 23-24, 2016.

Dr. Divya Chandran

1. Recipient of SERB Young Investigator Award, Department of Science and Technology, Government of India
2. Recipient of Innovative Young Biotechnologist Award, Department of Biotechnology, Government of India

Dr. Sivaram Mylavarapu

1. Reviewer for the International Journal of Biochemistry and Cell Biology

Publications

Original Peer-Reviewed Articles

1. Kottur J and Nair DT (2016) Reactive Oxygen Species play an important role in the bactericidal activity of Quinolone Antibiotics. Angew Chem Intl Ed Engl 55:2397.
2. Ghodke PP, Gore KR, Harikrishna S, Samanta B, Kottur J, Nair DT and Pradeepkumar PI (2016) The N(2)-furfuryl-deoxyguanosine adduct does not alter the structure of B-DNA. J. Org. Chem. 81:502.
3. Kumar A, Gupta C, Nair DT and Salunke DM (2016) MP-4 contributes to snake venom neutralization by *Mucuna pruriens* seeds through an indirect antibody-mediated mechanism. J. Biol. Chem. 291:11373.
4. Harshita, Chanchal and Jain D (2016) Cloning, expression, purification, crystallization and initial crystallographic analysis of FleN from *Pseudomonas aeruginosa*. Acta Cryst. F72, 135.
5. Jain D, Narayanan N and Nair DT (2015) Plasticity in repressor-DNA interactions neutralizes loss of symmetry in bipartite operators J. Biol. Chem. 291:1235.
6. Chaurasia P, Pratap S, von Ossowski I, Palva A and Krishnan V (2016) New insights about pilus formation in gut-adapted *Lactobacillus rhamnosus* GG from the crystal structure of the SpaA backbone-pilin subunit. Sci Rep. 6:28664.
7. Kant A, von Ossowski I, Palva A and Krishnan V (2016) Crystallization and X-ray Crystallographic Analysis of the Adhesive SpaC Pilin Subunit in the SpaCBA Pilus of Gut-adapted Lactobacillus rhamnosus GG. Protein & Peptide Letters 23:365
8. Rana S, Elci SG, Mout R, Singla AK, Yazdani M, Bender M, Bajaj A, Saha K, Bunz UHF, Jirik FR and Rotello VM (2016) Ratiometric Array of Conjugated Polymers-Fluorescent Protein Provides a Robust Mammalian Cell Sensor. J. Am. Chem. Soc.138:4522
9. Gupta S, Singh M, Reddy AM, Yavvari PS, Srivastava A, and Bajaj A (2016) Interactions governing the entrapment of anticancer drugs by low molecular weight hydrogelator for drug delivery applications. RSC Adv. 6:19751
10. Bhattacharya S, Hanpude P and Maiti TK (2015) Cancer associated missense mutations in BAP1 catalytic domain induce amyloidogenic aggregation: A new insight in enzymatic inactivation. Sci Rep 5:18462

11. Annarapu GK, Singhal R, Peng Y and Guchhait P (2016) Inhibition of Hb binding to GP1b-alpha abrogates Hb-mediated thrombus formation on immobilized VWF and collagen under physiological shear stress. Plos One, 11:e0154276.

12. Singhal R, Annarapu GK, Pandey A, Chawla S, Ojha A, Gupta A, Cruz MA, Seth T and Guchhait P (2015) Hemoglobin interaction with GP1balpha induces platelet activation and apoptosis: a novel mechanism associated with intravascular hemolysis. Haematologica, 100: 1526.

13. Da Q, Teruya M, Guchhait P, Teruya J, Olson JS and Cruz MA (2015) Free hemoglobin increases von Willebrand factor-mediated platelet adhesion *in vitro*: implications on circulatory devices. Blood. 126:2338.

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16. Meteignier, LV, Zhou, J, Cohen, M, Bhattacharjee, S, Brosseau, C, Chan, MG, Robatzek, S and Moffett, P (2016) NB-LRR signaling induces translational repression of viral transcripts and the formation of RNA processing bodies through mechanisms differing from those activated by UV stress and RNAi. J. Exp. Bot. 67 2353.

Reviews

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2. Chandran D and Wildermuth MC (2016) Modulation of host endocycle during plant-biotroph interactions. The Enzymes 40:65.

Patent

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Scientific Colloquium

For the benefit of the scientific community in the National Capital Region and coinciding with the Programme Advisory Committee meetings of the Centre, Colloquium Lectures are delivered by outstanding scientists from reputed institutions across the globe.

During the last PAC meeting a Colloquium lecture was delivered by Prof. Padmanabhan Balaram (Molecular Biophysics Unit, IISc). Prof. Balaram is an eminent Indian Biochemist and former Director of the Indian Institute of Science, Bangalore. He is a recipient of the Padma Bhushan award from the Government of India. His research interests include investigation of the molecular structure and biological activity of designed and natural peptides. On the occasion of the 6th Program Advisory Committee, Prof. Balaram delivered a lecture titled "Integrating Mass Spectrometry and Next Generation Sequencing in the Analysis of Cone Snail Venom Peptide Libraries". The lecture was attended by scientists from institutions in and around Delhi NCR.


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Extramural Activities and Funding

Extramural Activities and Funding

Multi-institutional Preterm Birth (PTB) Programme

A national level innovative multi-institutional study is being conducted through RCB, a key partner in the PTB study birth involving a very large cohort. The basic aim of the project is to understand the epidemiology of PTB, its genetic and environmental interactions, and changes in the vaginal microbial landscape. The highlights of the program include development and evaluation of putative biomarkers, identification of simple microbiological tool based vaginal risk factors, modulation of vaginal microbiota for therapeutic purposes and evaluation of environmental modification chosen from SNP analysis. Some of the major public health concerns addressed include biological risks and processes of foetal growth and PTB, clinical consequences of PTB and intra uterine growth retardation.

The PTB program actively involves bridging expertise from disparate fields, such as, paediatrics, gynaecology, infectious disease biology, epidemiology, microbiology, immunology, platform technologies, cellular & molecular biology, genetics, statistics and computational & systems biology. Applying a cross-disciplinary approach, it is proposed to elucidate possible mechanisms and outline the etiology of PTB. Whole-genome screens, study of genomics, epigenomics and proteomics in different time frames, will be performed to assess the biological risk factors and the dynamic nature of PTB. A metagenomic approach for profiling of vaginal microbial flora would be taken up and this information will be correlated with PTB and other dietary and epidemiological risk factors.

The long-term goal envisages clinically relevant research outputs that would aim to (i) achieve appropriate risk stratification of women early in pregnancy (ii) identify simple and better prediction tools that will recognize the optimal time of prediction & clinical intervention, (iii) develop additional strategies to identify presence of unusual/novel microbes that could serve as biomarkers, (iv) identify focused remedies targeting one or more mechanistic pathways (e.g. infection, inflammation, hormonal), (v) apply currently available interventions based on better understanding of biological mechanisms.

The personnel engaged in the project are comprising of a clinical team, multi-disciplinary scientific team and a project management team. The clinical team will be stationed at Gurgaon General Hospital, which is the site of the study, and comprises of the clinical coordinator, research physicians, nurses, attendants, field workers and field supervisors.

Proteomics analyses of the samples associated with this study are carried out at RCB. Proteomic analysis will provide information regarding the differentially regulated proteins and protein function alteration due to post translational modification of proteins in the preterm birth condition. The ultimate goal of RCB's approach is to decipher the molecular mechanisms contributing to the poor pregnancy outcome, based on proteomics studies.

Extramural Grants

1. Dr. C.V. Srikanth, "Understanding Salmonella-mediated alterations in host SUMOylation: implications in infection and inflammation", Wellcome Trust/ DBT India Alliance Intermediate Fellow (Rs. 328 lakhs).
2. Dr. Divya Chandran, "Deriving gene regulatory networks mediating legume host-powdery mildew pathogen cross-talk during compatible and incompatible interactions", Innovative Young Biotechnologist Award, Department of Biotechnology (Rs. 43.16 lakhs).
3. Dr. Divya Chandran, "Identification of novel regulators and nodes of response mediating powdery mildew sporulation on legumes", Early Career Research Award, Science and Engineering Research Board, Department of Science & Technology, (Rs. 39.11 lakhs).
4. Dr. Dinakar M Salunke (ICGEB), Dr. Deepak T. Nair (RCB), Dr. Deepti Jain (RCB), Dr. K. Vengadesan (RCB) & Dr. Tushar K. Maiti (RCB) "Data Driven Initiatives in Astronomy and Biology, A proposal for Joint Big Data Mining" jointly with Inter University Centre for Astronomy & Astrophysics, Principal Scientific Advisors Office (Rs. 300 lakhs).
5. Dr. Avinash Bajaj, "Engineering of self-assembled lipidated nanoparticles for cancer combination therapy" from Department of Science and Technology (Rs. 47.6652 Lakhs).
6. Dr. Avinash Bajaj, "Molecular engineering of low molecular weight injectable hydrogels with sustained drug release for cancer therapy" from Department of Biotechnology (Rs.76.36 Lakhs).
7. Dr. Deepti Jain, "Biochemical and Structural Characterization of the Single Polypeptide Mitochondrial RNA Polymerase - RpoTm", Early Career Research Award, Science and Engineering Research Board, Department of Science & Technology (Rs. 34.81 Lakhs).
8. Dr. Sam J Mathew, "Role of developmental myosin heavy chains in skeletal muscle development, regeneration, homeostasis and disease", Wellcome Trust/ DBT India Alliance Intermediate Fellow, (Rs. 350 Lakhs).
9. Dr. Puspha Kumari, "Understanding role of Exocyst complex in cell division and development in Caenorhabditis elegans", Welcome Trust/ DBT India Alliance Early Career Fellow, (Rs. 144 Lakhs).

10. Dr. Saikat Bhattacharjee, "Elucidating inositol-dependent signalling routes of effector-triggered immunity for identifying new approaches for engineering crop resistance against diverse pathogens", Ramalingaswami Fellow, Department of Biotechnology (Rs. 82 lakhs).

12. Dr. Deepti Jain, "Structure and mechanism of FleQ, master regulation of transcription of flagellar and biofilm genes in Pseudomonas aeruginosa." Innovative Young Biotechnologist Award 2015, Department of Biotechnology (Rs. 52 lakhs).

13. Dr. Dinakar Salunke and Dr. Tushar K Maiti, "Inter-institutional programme for Maternal, Neonatal and Infant sciences a Translational approach to studying PTB", Jointly with THSTI, NIBMG and General Hospital, Gurgaon, NII, and Safdarjung Hospital, Department of Biotechnology (Rs. 4885 lakhs).

14. Dr. Avinash Bajaj, "Engineering of Nanomaterials for Combination Cancer Therapy", Department of Biotechnology (Rs.30 lakhs).

15. Dr. Dinakar M Salunke, "Collaboration for translation and clinical research between Translation Health Science and Technology Institute, National Brain Research Centre, Regional Centre for Biotechnology and Gurgaon, Civil Hospital, Department of Biotechnology (Rs.79.05 lakhs).

16. Dr. Prasenjit Guchhait, "Pathophysiology of thrombocytopenia in dengue infection", Jointly with AIIMS, New Delhi, Department of Biotechnology (Rs. 55 lakhs).

17. Dr. Sivaram Mylavarapu,"Molecular Basis for Silencing of the Spindle Assembly Checkpoint", Department of Biotechnology (Rs. 35 lakhs).

18. Dr. Avinash Bajaj, "Phospholipid based Nanomaterials as Novel Therapeutics for Cancer", Department of Biotechnology (Rs. 29 lakhs).

19. Dr K Vengadesan, "Structural investigations of surface nano scale assembly in a gut bacterium", Department of Biotechnology (Rs. 60.0 lakhs).

20. Dr. Avinash Bajaj, "Design, Engineering, and Investigating the Anticancer/anti-angiogenic Properties of Bile Acid Amphiphiles for Colon Cancer Therapy" Jointly with NII, Department of Biotechnology. (Rs 60 lakhs).

21. Dr. Sam J Mathew, "The role of MET-CBL signaling in Rhabdomyosarcoma", Department of Biotechnology (Rs 24.54 lakhs).

22. Dr. Tushar K Maiti, "Targeting ubiquitin proteasome system for the anticancer drug development: A peptoid based inhibitor design, synthesis and evaluation", Department of Biotechnology (Rs 24.54 lakhs).

23. Dr. Deepak T Nair, "Effect of N2-adducts of deoxyguanosine of DNA synthesis by replicative and translesion DNA polymerases.", jointly with IIT-Bombay, Department of Biotechnology (Rs. 57 lakhs).

24. Dr. Avinash Bajaj, "Investigating the role of BLM helicase as a global tumour suppressor: understanding its regulatory loops and using the knowledge for therapeutic and clinical application in cancer biology", Department of Biotechnology (Rs. 80.9 lakhs).

26. Dr. Prasenjit Guchhait, "Understanding the distinct developmental and functional properties of the neonatal immune system and their clinical consequences in the neonatal period" jointly with THSTI; Department of Biotechnology (Rs. 183 lakhs).

27. Dr. Tushar Maiti, "Stress outcomes on pregnancy, fetal growth and birth weight: Development of methods to identify mothers at risk of preterm birth and intrauterine growth restriction resulting from maternal stress" jointly with NIBMG and THSTI; DBT-BMFG-BIRAC-USAID Award of Grand Challenge India-All Children Thriving. (Rs. 162 lakhs).

28. Dr. Deepak T Nair, "The role of DNA polymerase IV in ROS mediated lethality: Structure and Mechanism", Department of Biotechnology as part of National Bioscience Award for Career Development. (Rs. 15 lakhs).

International and National Networking

RCB-DAILAB

The Department of Biotechnology (DBT), through the Regional Centre for Biotechnology (RCB) and the National Institute of Advanced Industrial Science & Technology (AIST), through its Biomedical Research Institute (BRI), Japan had entered into a partnership for capacity building initiatives in bio-imaging and biotechnology in the year 2014. This initiative facilitates enhancement of career opportunities for scientists and researchers working in biomedical, clinical, and other related areas of biotechnology and complements the existing bilateral research cooperation between the Govt. of India and the Govt. of Japan.

A DBT-AIST joint lab (DAILAB) for advanced research training in bio-imaging has been established at RCB. The DAILAB will facilitate joint research collaborations engaging Indian and Japanese scientists and support selected Indian researchers for training in specialized areas of bio-imaging and biotechnology, both in Japan and in India.

The new collaboration provides an opportunity for both the institutions in capacity building, training and research collaborations and will benefit young scientists not only in India and Japan but also from the UNESCO member countries in the Asia-Pacific and SAARC regions. Indeed, through the current initiatives, RCB, an institution engaged in broad-based multidisciplinary training, education and research is poised for broadening its horizons and will facilitate bridging science and knowledge dissemination for the betterment of mankind.

BM14 Project

In late 2008 an agreement was signed between Department of Biotechnology, European Molecular Biology Laboratory (EMBL) and European Synchrotron Radiation Facility (ESRF) that enabled the Indian scientists to visit the BM14 beamline located in ESRF to collect X-ray diffraction data. This project began operations in the spring of 2009 and initial sanction was for five years. Since its inception, the project has facilitated the research of hundreds of scientists from all over India and has resulted in a number of publications in leading international journals. In late 2014, the project was extended for a further period of two years and the management of the project was moved from NII to RCB. A tripartite agreement was signed between RCB, EMBL and ESRF to facilitate the extension of this project for the period 2014-2016. The BM14 project has allowed the Indian researchers to publish more than 200 papers in prestigious international journals and enabled training of more than 150 PhD students in macromolecular crystallography.

NCR Biotech Science Cluster, Faridabad

RCB is an integral founding partner of the NCR Biotech Science Cluster (BSC) at Faridabad in the national capital region (NCR) of Delhi. The BSC supports discovery of basic mechanisms in biology and development of novel technologies and reagents associated with biotechnology. The cluster aims to facilitate public-private partnerships for the development of biotechnology business incubators and technology parks, including creative partnerships with Biotech & Pharma entrepreneurs and distributors. The cluster will network with the potential constituent institutions to create a synergistic ecosystem for accelerating discoveries and facilitating translation of these discoveries to real world solutions in the field of health care and agriculture and their commercialization. In terms of infrastructure, the cluster will have a state-of-the-

art technology platform center, animal facilities and bio safety containment laboratories.

Advanced Technology Platform Centre (ATPC)

The state-of-the-art Advanced Technology Platform Centre (ATPC) is established within the centre as a central equipment repository. The ATPC would act as a catalyst for multidisciplinary basic and translational research and development by providing relevant instrumentation, training and professional services for the stakeholders and others alike on behalf of the NCR Biotech Science Cluster in Faridabad. The ATPC is an initiative for multidisciplinary research that translates scientific and technological advancements into innovations that will improve public health.

NCR Biotechnology Business Incubator

The biotechnology business Incubator is being established as part of the NCR Biotech Science Cluster in partnership with BIRAC. This state-of-the-art facility would provide new and emerging companies with conducive environment that would support their start-up phase and increase their likelihood of success. The incubator aims to cater to the needs of companies acquiring technology from abroad for soft landing and also provide facilities for pilot scale lot production under GMP for new products. In addition, the incubator will also facilitate prototype to product conversion for devices and implants. The planned incubator includes facility space, flexible leases and shared use of common office.

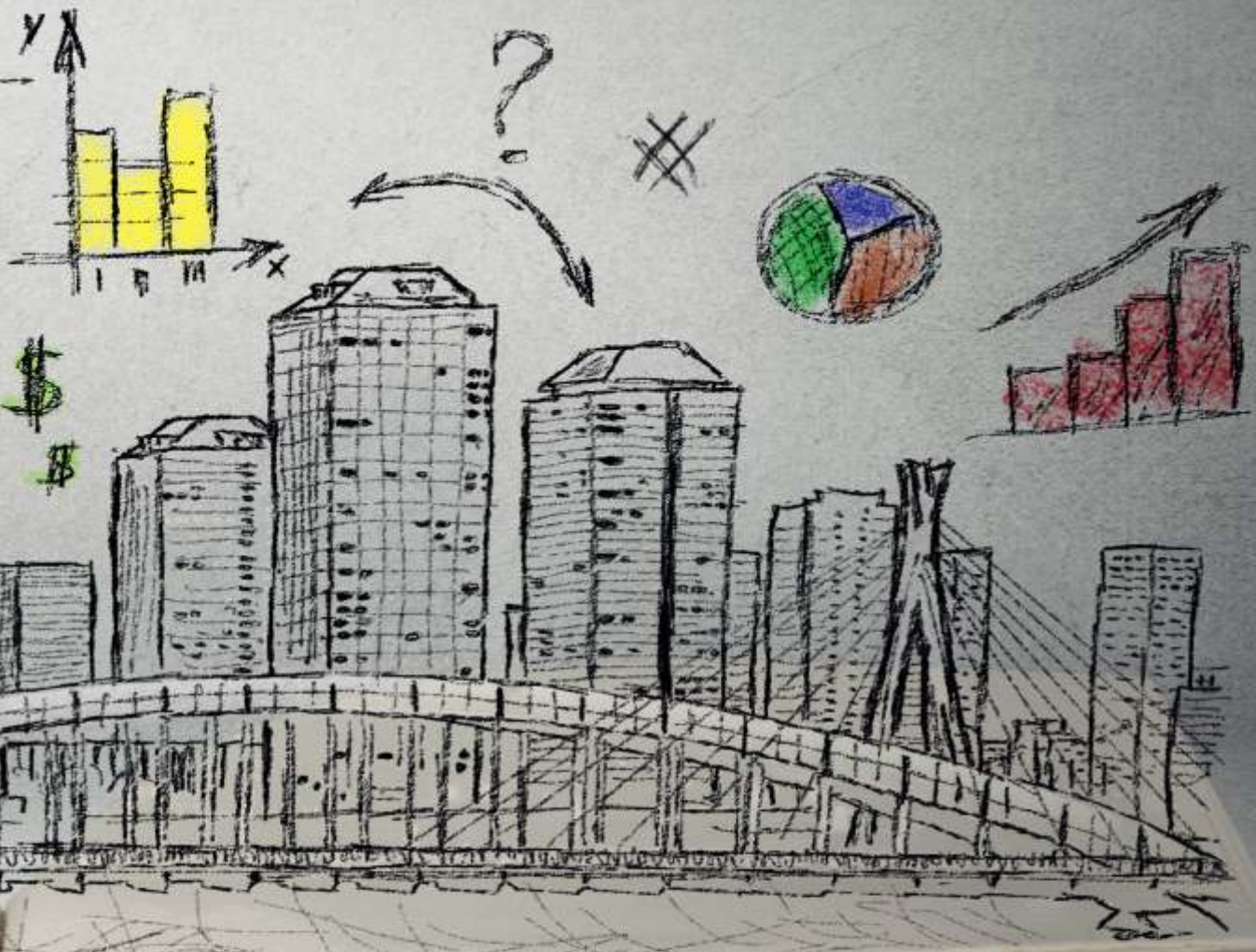
Technology Advancement Unit

Technology Advancement Unit (TAU) is a joint initiative of the Swiss Agency for Development and Collaboration and the Department of Biotechnology. The Technology Advancement Unit is being set up with the aim to create a conductive environment for the planning and implementation of R&D projects, focussed on product development/technology transfer thereby promoting technology advancement and transfer in the development context. RCB is managing the Technology Advancement Unit as part of its multi-dimensional role of an inter-institutional coordinator. The TAU is setup with the objective to create an effective ecosystem for technology transfer and product development. Thus, TAU acts as a facilitator, performing an advisory function and providing support through active exchange of information with key stakeholders including academic institution, government and industries.

Biosafety Support Unit

RCB has been entrusted by the Department of Biotechnology, Ministry of Science and Technology, Govt. of India the responsibility for providing administrative and financial support to the Biosafety Support Unit (BSU) which will develop guidelines and protocols for generating biosafety data to address the national challenges raised by the emerging areas of biotechnology. The Biosafety Unit would streamline the functioning of the Review Committee on Genetic Manipulation/ Genetic Engineering Approval Committee. The unit would provide appropriate scientific information on emerging issues and, in addition, a Journal of Biosafety Regulation would also be published for knowledge dissemination in this important area.

Infrastructure



Laboratory Infrastructure

RCB is equipped with the state-of-the-art infrastructure for conducting research, education and training in modern areas of biology and biotechnology. The facilities available include:

Microscopy and Imaging: The facility houses a confocal microscope, a fluorescence microscope, an atomic force microscope, a laser capture microdissection microscope, an infrared imager and a chemiluminescence imager.

Macromolecular Crystallography: This facility has an automated nanodispenser for crystallization, UV and light microscopes, X-ray generator with optics, detector and cryostream.

Proteomics: Mass Spectrometers, HPLC, Nano LC spotter, 2-D gel electrophoresis system and a protein sequencer are part of this facility.

FACS: A high-end FACS analyzer is available for cell counting and biomarker detection.

Nuclear Magnetic Resonance/(NMR): A 400 MHz NMR Spectrometer is also equipped with a broadband probe, cryo and variable temperature probes to facilitate different applications.

The Common Instrument Facilities (CIFs) have a number of instruments for probing molecular interactions. These include Surface Plasmon Resonance Unit, Isothermal Titration Calorimetry Unit, Differential Scanning Calorimetry system, Multipurpose Plate Readers, Dynamic Light Scattering Instrument, UV spectrophotometer, IR spectrophotometer, Fluorimeter and CD Spectro-polarimeter. In addition, equipment such as a laser scanner for biomolecular imaging, gel documentation units, RT-PCR machine and nanodrop spectrophotometer are also available in the CIFs.

A number of instruments for preparation of samples also exist for research and training purposes. These include plant growth chambers, cell-culture facility, laminar flow hoods, chemical hoods, high speed & high volume floor centrifuges, benchtop centrifuges, emulsifier, sonicator, tissue homogenizer, shaker-incubators, microwave tissue processor, tissue embedding station, microtome, water-baths, PCR machines, electroporator, water purification systems, autoclaves, ice machines and cold rooms.

The Advanced Technology Platform Centre is currently housed within the RCB premises and three facilities within ATPC are already up and running. The Fluorescence activated cell sorting (FACS), Protein Purification and Mass Spectrometry Facilities are used by researchers from different institutes associated with the Cluster. The ATPC building will be ready soon and will be populated with facilities that will provide researchers with access to cutting-edge technologies.

The DBT-AIST International Laboratory for Advanced Bio-Imaging (DAILAB) is a joint collaboration between RCB and Advanced Industrial Science & Technology (Japan). The DAILAB currently houses a state-of-the-art *in vivo* imager, fluorescence and stereo- microscopes and a multi-mode plate reader.

In addition, a Small Animal Research Facility and a Plant Research Facility are also functional and available for researchers in RCB. In the near future, a modern BioSafety Level 3 (BSL3) facility will be built to support research on pathogenic organisms conducted by RCB scientists.

Each spacious laboratory here is shared between Principal Investigators (PIs). The laboratories have work and lab preparation benches, storage furniture, seating space for research members equipped with computers, networked PI cabins along with internet and phone access. All laboratories are equipped to prepare samples which are primary for conducting research in their specialized areas. Specialized facilities such as cold room, dark room, X-ray rooms have been set for undertaking specialized experimental research.

The Centre has rooms reserved for laboratory meetings, interactions, discussions, teaching and tutorials. The auditorium complex of the NCR Biotech Science Cluster includes a 400-seater auditorium and two seminar rooms (each with a seating capacity of 150).

The auditorium complex is utilized for conducting meetings, seminars, workshops and conclaves.

Digital Initiatives

The Information and Communication facilities at RCB are continuously evolving and an array of information technologies and resources have currently been deployed in the campus. These include Computing Facilities for RCB staff and students and about 150 client machines with different operating systems. Various off-the-shelf software are available in RCB to fulfill the requirements of the users and ensure security of the network and computer. Biometric Attendance System (BAS) has also been enabled for the staff, to register attendance by simply presenting his/her biometric (finger print). A competent & experienced IT service support team has been put in place to develop a user-friendly and dynamic web-site (www.rcb.res.in) that provides current information about all the activities in RCB. The e-mail system at RCB offers a web based e-mail that allows on-campus and remote access to emails. Additionally the executives and staff have email IDs on the National Informatics Centre servers, in compliance to the E-mail policy of the Government of India. Regarding the internet connectivity, RCB has 100 Mbps shared internet leased line from the National Knowledge Network offering high speed internet connectivity in the campus. The auditorium, conference and seminar halls are equipped with sound and projection systems, digital podium and internet connectivity. RCB has an internet based Video Conferencing facility setup in the seminar hall that can be used for holding virtual seminars or conferences.

The Centre has been functioning in conformity with the guidelines of the Government of India with regard to guidelines on IPV6 implementation and has also been an active participant in the Government initiatives of the "Digital India Campaign".

RCB Library

The library & e-library facility have been fully established with regular subscription of print and electronic versions of scientific journals. The Library has more than 500 textbooks spanning various areas of biotechnology for teaching and research. The e-library has online access to more than 1171 e-journals via the DBT Electronic Library Consortium (DELCON). The RCB library is integrated with Libsys library management system for library operations. Web-OPAC facility is also available for online searches of library documents. The library conducts a user awareness/training program for the new students every year.

Financial Statements

SRIVASTAVA KUMAR & CO.
CHARTERED ACCOUNTANTS
21-A NANGLI RAZAPUR
SARAI KALE KHAN, NIZZAMUDIN EAST
NEW DELHI-110013

INDEPENDENT AUDITOR'S REPORT

We have audited the attached balance sheet of REGIONAL CENTRE FOR BIOTECHNOLOGY, 3rd **Milestone, Faridabad - Gurgaon Expressway, Faridabad** as on 31st March, 2016 and its Income and Expenditure Account for the year ended on annexed thereto. These Financial Statements are the responsibility of the centre's management. Our responsibilities express an opinion on these financial statements based on our audit.

1. That the centre's balance sheet, Income & Expenditure Account and Receipt and Payment Account Agreement with the books of accounts.

2. We conducted our audit in accordance with auditing standard generally accepted in India. Those standards require that we plan and perform the audit to obtain reasonable assurance about whether the statements are free of material misstatement. An audit includes examining, on a test basis, evidence supporting the amounts and disclosures in the financial statements. An audit also includes assessing the accounting principles used and significant estimates made by the management, as well as evaluating the overall statement presentation. We believe that our audit provides a reasonable basis for our opinion.

3. Subject to accounting policies and notes on accounts as per schedule-10, in our opinion and to the best information and according the explanation give to us, the said accounts give a true and fair view:

(a) In Case of Balance Sheet, of the statement of affairs of the centre as at 31.03.2016 and

(b) In case of Income Tax & expenditure account, of the centre during the period ended on 31st March 2016

As per our Audit Report of Even dated
For: SRIVASTAVA KUMAR & CO.
CHARTERED ACCOUNTANTS
FRN: 011204N

RASHMI GUPTA
(PARTNER)
M.NO. 526817
PLACE: NEW DELHI
DATED: 29/09/2016

REGIONAL CENTRE FOR BIOTECHNOLOGY
BALANCE SHEET AS AT 31ST MARCH, 2016

Amount [in Rs.]

CORPUS / CAPITAL FUND AND LIABILITIES	Schedule		Current Year		Previous Year
CAPITAL GRANTS FOR INFRASTRUCTURE	1	-	18,38,71,920		10,83,15,017
RESERVES AND SURPLUS	2	-	4,15,012		4,15,012
CURRENT LIABILITIES AND PROVISIONS	3(A)	-	15,26,49,240		1,21,84,713
BIOTECH SCIENCE CLUSTER (DBT)	3(B)	-	2,11,14,53,006		1,99,94,83,798
TOTAL			**2,46,04,23,184**		**2,12,08,27,540**
ASSETS					
FIXED ASSETS	4		12,67,12,719		13,52,35,912
FUNDS IN SUBSIDIARY DEPOSITS	5(B)		4,28,92,000		5,48,25,000
CURRENT ASSETS,LOANS ADVANCES ETC.	5(A+C)	-	33,68,71,695		34,76,96,630
BIOTECH SCIENCE CLUSTER (DBT)	5(D)		1,91,18,95,690		1,60,30,58,940
a. Capital Work in progress	4	1,02,06,73,383		1,21,47,66,254	
b. Advance to BSC construction	5(C II & III)	24,45,37,381		26,57,93,658	
c. Funds in short term deposits	5 (D i)	34,00,000		34,00,000	
d. Accrued Interest & TDS	5(i iv & v)	44,16,774		61,78,028	
TOTAL			**2,46,04,23,184**		**2,12,08,27,540**
SIGNIFICANT ACCOUNTING POLICIES & NOTES TO ACCOUNTS					

AS PER OUR SEPARATE REPORT OF EVEN DATE ATTACHED
M/s Subrata Kumar and co.
Chartered Accountants

BIJU MATHEW
SENIOR MANAGER (F&C)

SUBRATA SINHA
EXECUTIVE DIRECTOR

RASHMI GUPTA
PARTNER

PLACE: Faridabad
Date 29/09/2016

REGIONAL CENTRE FOR BIOTECHNOLOGY

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31st MARCH, 2016

Amount (In Rs.)

INCOME	Schedule	Current Year	Previous Year
Grants/Subsides	6	14,99,22,311	13,48,06,602
Fees/Subscriptions	7(i & iii)	3,73,920	2,27,720
Interest on investments on fixed deposits/savings a/c	7(ii)	36,26,788	48,05,118
Deferred Income-Fixed Assets	1	6,24,40,221	6,63,62,005
TOTAL (A)		**21,62,63,240**	**20,62,01,535**
EXPENDITURE			
Establishment Expenses	8	6,42,01,783	4,89,71,484
Other Administrative Expenses etc.	9	11,23,42,321	9,18,60,107
Excess of Expenditure Carried over	5(c 7)	(2,27,21,085)	(9,92,061)
Depreciation (Net Total at the year end)	4	6,24,40,221	6,63,62,005
TOTAL(B)		**21,62,63,240**	**20,62,01,535**
Balance being excess of Income Over Expenditure (A-B)		-	
Transfer to special Reserve(Specify each)			
Transfer to /from General Reserve		-	
BALANCE BEING SURPLUS (DEFICIT) CARRIED TO CORPUS/CAPITAL FUND		-	

AS PER OUR SEPRATE REPORT OF EVEN DATE ATTACHED
M/s Srivastava Kumar & Co
Chartered Accountants

BIJU MATHEW
SENIOR MANAGER (A&F)

SUBRATA SINHA
EXECUTIVE DIYECTOR

RASHMI GUPTA
PARTNER

PLACE: Faridabad
DATE: 29/09/2016

Srivastava Kumar & Co. NEW DELHI Chartered Accountants

Regional Centre for Biotechnology

Accounting Policies and Notes Forming Parts of the Balance Sheet and Income & Expenditure Account for the Year Ended at 31st March, 2016

1. The annual accounts have been prepared in the revised format of accrual system of accounting.

2. The Centre has been registered as a Society under the Haryana Regulation and Registration of Societies Act 2012 on 9th February 2015 and existed previously based on the Executive Order of the Government. Furthermore, the bill for enactment of RCB by the Parliament is under process as on 31.3.2016. Hence the accounts of the Centre has been drawn up with the incorporated fact that the assets and liabilities of the existing entity has been transferred to the Society and have been taken over by the Society on the date of formation.

3. Since the RCB bill has been passed, as on the date of signing the audit report, the liabilities on account of provisions for Gratuity & terminal benefits of the Centre will worked out & incorporated in the accounts for FY 2016-17 in accordance with the approved service conditions of the RCB to be adopted. No provision has been made by the Institute towards the gratuity payable and other terminal benefits to staff during the FY 2015-16.

4. (a) Recurring Grants have been recognised in the Income & Expenditure account and non recurring Grants have been shown as part of capital. The final Utilization Certificate to be submitted to the Department of Biotechnology on the basis of the audited accounts.

   (b) Grants for core funds relatable to depreciable fixed assets are treated as deferred income and recognised in the Income and Expenditure Account on a systematic and rational basis over the useful life of such assets i.e. such grants are allocated to income over the periods and in the proportions in which depreciation in charged. During the year income recognised in respect of such Grants amounts to Rs. 624,40,221

5. (a) The depreciation has been provided w.e.f. the date of installation/put to use of fixed assets as per the rates prescribed by Income Tax Act 1961. During the previous year depreciation has been charged at per rate prescribed.

   (b) Depreciation has been charged during the year of acquisition and no depreciation is provided during the year of assets sold / discarded.

Srivastava Kumar & Co. NEW DELHI Chartered Accountants

6. Fixed assets have been created with grants received from the Department of Biotechnology. The condition of these grants, inter- alia, stipulates that assets will be the property of Government, who will be free to sell or otherwise dispose off the same. The Govt. of India has the discretion to gift the assets to the Institute if it considers appropriate, but no such gifts have been made so far. Therefore, in effect the ownership of the assets lies with Govt. of India and not with the Institute.

7. All purchases of chemicals, glassware, consumables and stationary have been charged to consumption at the time of purchase without working out closing stock at the end of the year.

8. Further all entries relating to purchase of consumables /equipments or other fixed assets in accounts are being passed only at the time of submission of satisfactory inspection/installation report irrespective of the date of actual receipt of the supplies / equipments.

9. In the absence of paid bills or copies of vouchers, Expenses and Overheads incidental to construction of building of institute, as reported by the Project Monitoring Consultant (Engineers India Limited), are added to the capital work in progress to be capitalized along with the building, only on submission of bills by the PMC. The project is being operated with an agreement which stipulates operation of an Escrow Account by NCR Biotech Science Cluster and has been opened in the Oriental Bank of Commerce, Bhikaji Cama Place. The Account Number is 03691011009170 and the authorized signatories are Engineers India Ltd. (Project Management Consultant)

10. The Institute has a policy of allocating the overheads and transfer of expenditure from institute to different projects at the end of year on proportionate basis after taking into account the amount of maximum permissible limits for overheads sanctioned by the funding agency in each project. During the year institute has allocated Rs. 18,52,444 , as overheads to different projects.

11. The institute has a policy of incurring expenditure on various projects in accordance with the sanctioned budget under various heads of accounts irrespective of the actual releases during a financial year. Since the actual release of money by the sponsoring agency is subject to various factors, the expenditure on approved heads of accounts is being incurred within the overall sanction of the project.

12. The balances of the previous year have been rearranged as per requirement and shown in Balance Sheet against the relevant heads.

13. The Institute has received contribution of Rs. 20371,59,968 (including RCB) from various institutes for the under Phase I & Phase I (Extension) of the construction of campus at Faridabad. The consolidated details are as under:

Srivastava & Co. NEW DELHI Chartered Accountants

Rs. In lakhs)

Sl.No	Constituent Partner	Opening Balance as on 1.4.2015	Received during 2015-16	Total receipts on 31.3.2016
1	THSTI	7883.30	1500.00	9383.30
2.	NII	1879.02	0.00	1879.02
3.	RCB	5500.65	1000.00	6500.65
4.	Bio-Incubator	1304.00	16.16	1320.16
5.	ATPC	50.00	527.22	577.22
6.	Interest on investment of BSC funds	625.91	85.34	711.25
	Total	**17242.88**	**3128.72**	**20371.60**

and the total expenditure incurred as on 31st March 2016 against such contribution is amounted to Rs. 193,15,30,966 (Rs.168,66,73,385 being the booked as Capital Work-in-progress and & Rs. 24,48,57,581 being advanced to the Project Monitoring Consultant).

14. The Capital Work-in-progress booked in the accounts includes the already constructed laboratory buildings of THSTI, RCB & NII and the under construction(68 % approx completed) buildings of ATPC, Bio-incubator, the hostels & faculty housing and common facilities like the Engineering services, the roads, the electrical installations, the sewerage treatment plant etc. The constituent wise allocation of expenditure & capitalization of assets including common facilities will be done on closure of the project, in accordance with the formal agreement made by the constituent partners.

For Srivastava Kumar & Co.

Chartered Accountants

(Biju Mathew)
Sr. Manager (A&F)

(Dr. Subrata Sinha)
Executive Director

Rashmi Gupta
Partner

Srivastava Kumar & Co. NEW DELHI Chartered Accountants

Place: Faridabad
Date: 29/09/2016

Institutional Governance

Board of Governors

1.	Prof. K. VijayRaghavan Secretary Department of Biotechnology, Ministry of Science & Technology, Govt. of India New Delhi	Chairperson (Ex-officio)
2.	Mr. Shigeru Aoyagi Director & UNESCO Representative to Bhutan, India, Maldives and Sri Lanka UNESCO Office New Delhi	Member (Ex-officio)
3.	Dr. Niranjan Chakraborty Director National Institute of Plant Genome Research New Delhi	Member (Ex-officio)
4.	Prof. Sudhanshu Vrati Executive Director Regional Centre for Biotechnology NCR Biotech Science Cluster Faridabad	Convener (Ex-officio)
5.	Dr. Sundeep Sarin Director & RCB Nodal Officer Department of Biotechnology Ministry of Science & Technology, Govt. of India New Delhi	Special Invitee (Ex-officio)

Programme Advisory Committee

1.	Prof. Angelo Azzi Tufts University Boston, USA	Chairperson
2.	Prof. Subrata Sinha Director National Brain Research Centre Manesar	Member
3.	Dr. Satyajit Rath Senior Scientist National Institute of Immunology New Dellhi	Member
4.	Prof. K. Veluthambi School of Biotechnology, Madurai Kamaraj University Madurai	Member
5.	Dr. Sundeep Sarin Director & RCB Nodal Officer Department of Biotechnology Ministry of Science & Technology, Govt. of India New Delhi	Member (Ex-officio)
6.	Dr. K. V. S. Rao Translational Health Science & Technology Institute NCR Biotech Science Cluster Faridabad	Member
7.	Dr. Gangandeep Kang Executive Director Translational Health Science & Technology Institute NCR Biotech Science Cluster Faridabad	Member (Ex-officio)

8.	Prof. T. P. Singh Department of Biophysics All India Institute of Medical Sciences (AIIMS) New Delhi	Member
9.	Prof. Joel L. Sussman Department of Structural Biology The Weizmann Institute of Science Rehovot, Israel	Member
10.	Prof. Keiichi Namba Graduate School of Frontier Biosciences Osaka University Osaka, Japan	Member
11.	Prof. R. Venkata Rao Vice Chancellor National Law School of India University Bangalore	Member
12.	Prof. Sudhanshu Vrati Executive Director Regional Centre for Biotechnology NCR Biotech Science Cluster Faridabad	Member (Ex-officio)
13.	Prof. Akhilesh K. Tyagi Department of Plant Molecular Biology University of Delhi, South Campus New Delhi	Special Invitee
14.	Dr. Dinakar M. Salunke Director International Centre For Genetic Engineering And Biotechnology New Delhi	Special Invitee

Executive Committee

1.	Prof. Sudhanshu Vrati Executive Director Regional Centre for Biotechnology NCR Biotech Science Cluster Faridabad	Chairperson (Ex-officio)
2.	Mr. Shigeru Aoyagi Director & UNESCO Representative to Bhutan, India, Maldives and Sri Lanka UNESCO Office New Delhi	Member (Ex-officio)
3.	Mr. Rakesh Ranjan Joint Secretary Ministry of Human Resource Development Govt. of India New Delhi	Member (Ex-officio)
4.	Mr. Manish Chauhan Joint Secretary (UNES) Ministry of External Affairs Govt. of India New Delhi	Member (Ex-officio)
5.	Dr. Sundeep Sarin Director & RCB Nodal Officer Department of Biotechnology Ministry of Science & Technology, Govt. of India New Delhi	Member (Ex-officio)
6.	Dr. Satyajit Rath Senior Scientist National Institute of Immunology New Delhi	Special Invitee

Finance Sub-Committee

1.	Prof. Sudhanshu Vrati Executive Director Regional Centre for Biotechnology NCR Biotech Science Cluster Faridabad	Chairperson (Ex-officio)
2.	Dr. Gagandeep Kang Executive Director Translational Health Science & Technology Institute NCR Biotech Science Cluster Faridabad	Member (Ex-officio)
3.	Smt. Sumita Mukherjee Joint Secretary & Financial Advisor Department of Biotechnology Ministry of Science & Technology, Govt. of India New Delhi	Member (Ex-officio)
4.	Dr. Sundeep Sarin Director & RCB Nodal Officer Department of Biotechnology Ministry of Science & Technology, Govt. of India New Delhi	Member (Ex-officio)
5.	Dr. Satyajit Rath Senior Scientist National Institute of Immunology New Delhi	Member

Scientific Personnel

Faculty

Executive Director
Prof. Sudhanshu Vrati

Professor
Dr. Prasenjit Guchhait

Associate Professors
Dr. Deepak T. Nair
Dr. Avinash Bajaj
Dr. Sivaram V. S. Mylavarapu
Dr. Tushar Kanti Maiti
Dr. Vengadesan Krishnan
Dr. Chittur V. Srikanth

Assistant Professors
Dr. Sam Jacob Mathew
Dr. Deepti Jain
Dr. Saikat Bhattacharjee
Dr. Divya Chandran

J.C Bose National Fellow
Dr. Dinakar M. Salunke

Emeritus Scientist
Prof. S. V. Eswaran

International Adjunct Faculty
Prof. Falguni Sen

Young Investigators
Dr. Amit Kumar Yadav
Dr. Masum Saini
Dr. Prabhakar
Dr. Shivendra Pratap
Dr. Shreeshail Sonyal
Dr. Rashi Gupta
Dr. Sheetal Chawla
Dr. Suneel Kumar Tripathi
Dr. Vaibhav Kumar Pandya
Dr. Bharat Singh
Dr. Manoj Kumar Patel
Dr. Siddhi Gupta
Dr. Megha Kumar

WT-DBT IA Early Career Fellow

Dr. Pushpa Kumari

Senior Research Fellows

Ms. Abha Jain
Mr. Sagar Mahale
Mr. Vedagopuram Sreekanth
Mr. Harsh Kumar
Ms. Harmeet Kaur
Ms. Pranita Hanpude
Mr. Pergu Rajaiah
Mr. Gowtham Kumar Annarapu
Mr. Somnath Kundu
Ms. Gayatree Mohapatra
Mr. Roshan Kumar
Mr. Amit Sharma
Ms. Priyanka Chaurasia
Ms. Sarita Chandan Sharma
Mr. Salman Ahmad Mustfa
Ms. Rashi Singhal
Ms. Chanchal
Ms. Kavita Yadav
Ms. Hitika Gulabani
Ms. Angika Bhasym
Ms. Megha Agarwal
Ms. Tanu Johari
Ms. Amrita Kumari
Ms. Abhiruchi Kant
Mr. Nihal Medatwal
Mr. Sanjay Kumar
Mr. Pankaj Kumar
Mr. Sandeep Kumar
Ms. Amrita Ojha
Ms. Sarika Rana
Ms. Sheenam
Mr. Manhar Singh Rawat
Mr. Jithesh Kottur
Mr. Naveen Narayanan
Mr. Sanjay Pal
Ms. Sunayana Dagar
Mr. Abhin Kumar Megta
Ms. Akashi
Ms. Sulagna Bhattacharya
Ms. Meha Shikhi
Ms. Priyajit Banerjee
Mr. Syed Mohd.Aamir Suhail
Ms. Rajnesh Kumari Yadav
Mr. Ingole Kishore Dnyaneshwar
Ms. Megha Gupta
Mr. Pharvendra Kumar
Ms. Minakshi Sharma
Ms. Raniki Kumari
Mr. Rahul Sharma
Ms. Shivlee Nirwal
Mr. Deepankar Singh
Ms. Shilpi Nagpal
Ms. Mary K Johnson

Junior Research Fellows

Ms. Hridya Chandrasekar
Ms. Shreyasi Das
Ms. Manisha Kumari
Ms. Arunima Gupta
Mr. Krishnendu Goswami
Mr. Mritunjay Kasera
Mr. Zaid Kamal Madni
Mr. Chandan Kumar
Ms. Swatee Sabri Upadhyay
Ms. Akriti Sharma
Mr. Srimali Nishith Maheshbhai
Ms. Sandhini Saha
Mr. Animesh Kar
Ms. Anushree
Ms. Jyotsna Misra
Mr. Asra Nasir Khan
Ms. Preksha Gaur
Ms. Sonalika Maurya
Ms. Priyanka Verma
Ms. Shraddha Dahale Kantilal
Mr. Saibal Saha
Mr. Pankaj Kumar
Ms. Vaishali Uniyal
Mr. Patterson Clement

Research Associates/ Post-Doctoral Fellows

Dr. Mukesh Kumar
Dr. Jewel Jameeta Noor
Dr. Deepak Kumar Jangir
Dr. Amit Kumar Dey
Dr. Arjun Kumar Misra
Dr. Bhoj Kumar
Dr. Mrittika Sengupta
Dr. Tapas Bhattarcharya
Dr. Gunjan Sharma
Dr. Sameer Gupta

Project Assistants/Fellows

Ms. Neha Sharma
Ms. Nidhi
Mr. Abhishek Kumar Singh
Mr. Pankaj Kumar Sahoo
Ms. Deepali Chhoker
Mr. Tuhin Subhra Haldar
Mr. Devesh Kumar Chaukikar
Mr. Shlok Jindal
Mr. Malyla Vamshikrishna
Ms. Aakanksha Verma
Ms. Neha

Senior Technology Officer

Mr. Ashish Kumar

Scientific Officer - Projects

Mr. Suneel Prajapati

Institute Management

Executive Director
Prof. Sudhanshu Vrati

Technical Assistant to Executive Director
Mr. Ramesh Chandiramouli

Academics

Registrar
Dr. B. Chandrasekar

Documentation Assistants
Mr. Deepak Kumar
Ms. Vaishali Mangla

Administration & Finance

Executive Director
Prof. Sudhanshu Vrati

Senior Manager (A&F)
Mr. Biju Mathew

Administrative Officer
Mr. V. M. S. Gandhi

Section Officer
Mr. Rakesh Yadav

Management Assistants
Mr. Sanjeev Kumar Rana
Mr. Sudhir Kumar

Technical Officer

Mr. Mahfooz Alam

Technical Assistants

Mr. Madhava Rao Medikonda
Ms. Vishakha Chaudhary
Mr. Suraj Tewari
Mr. Atin Jaiswal
Mr. Vijay Kumar Jha
Mr. Ramesh Chandiramouli
Mr. G. Nagavara Prasad
Mr. Kamlesh Satpute

Engineering

Executive Engineer

Mr. Ramesh Kumar Rathore

Junior Consultant

Mr. A. K. Singhal

Consultants

Science and Technology

Dr. Nirpendra Singh

Junior Consultant

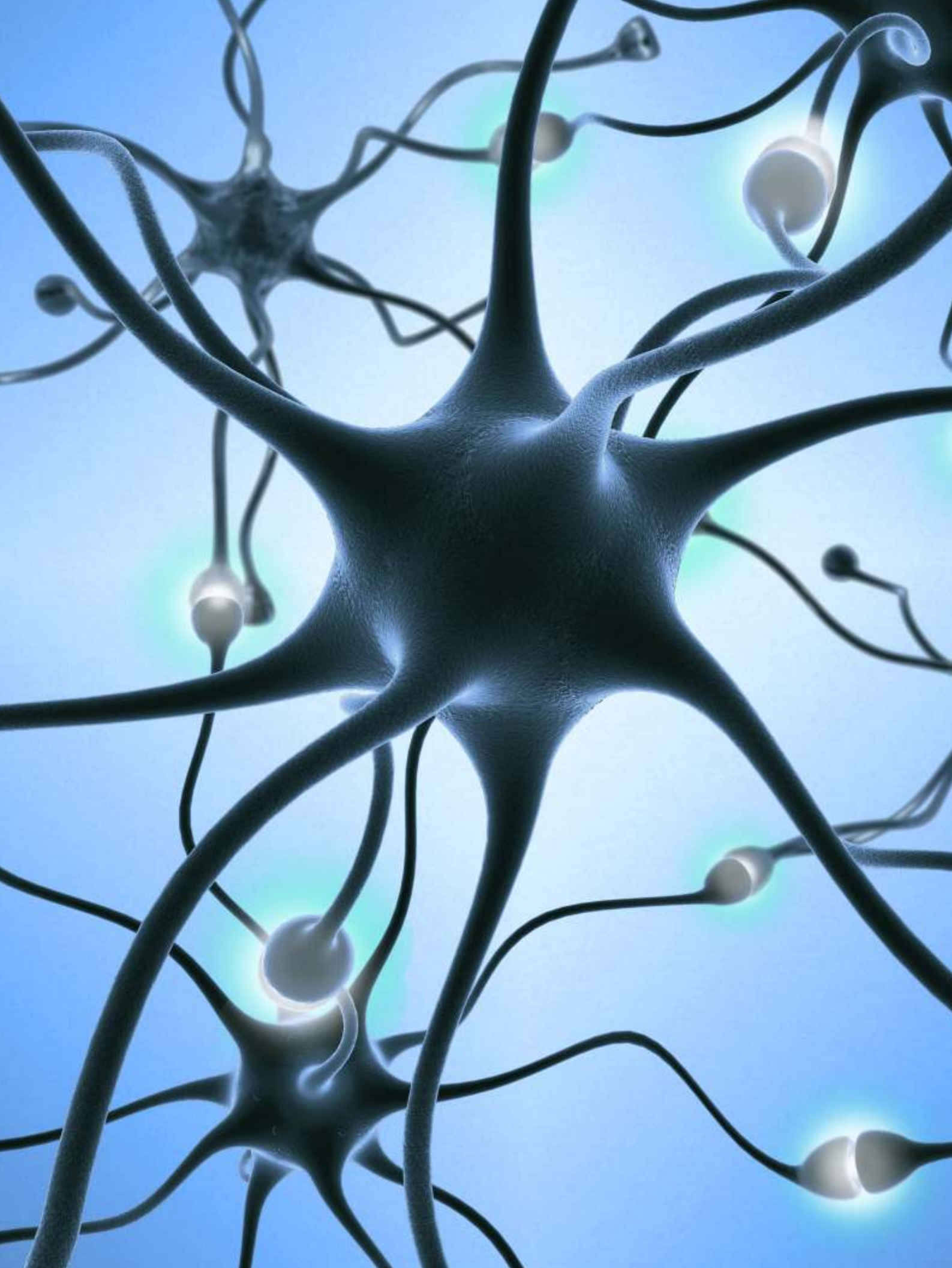
Ms. Nikita Siwach

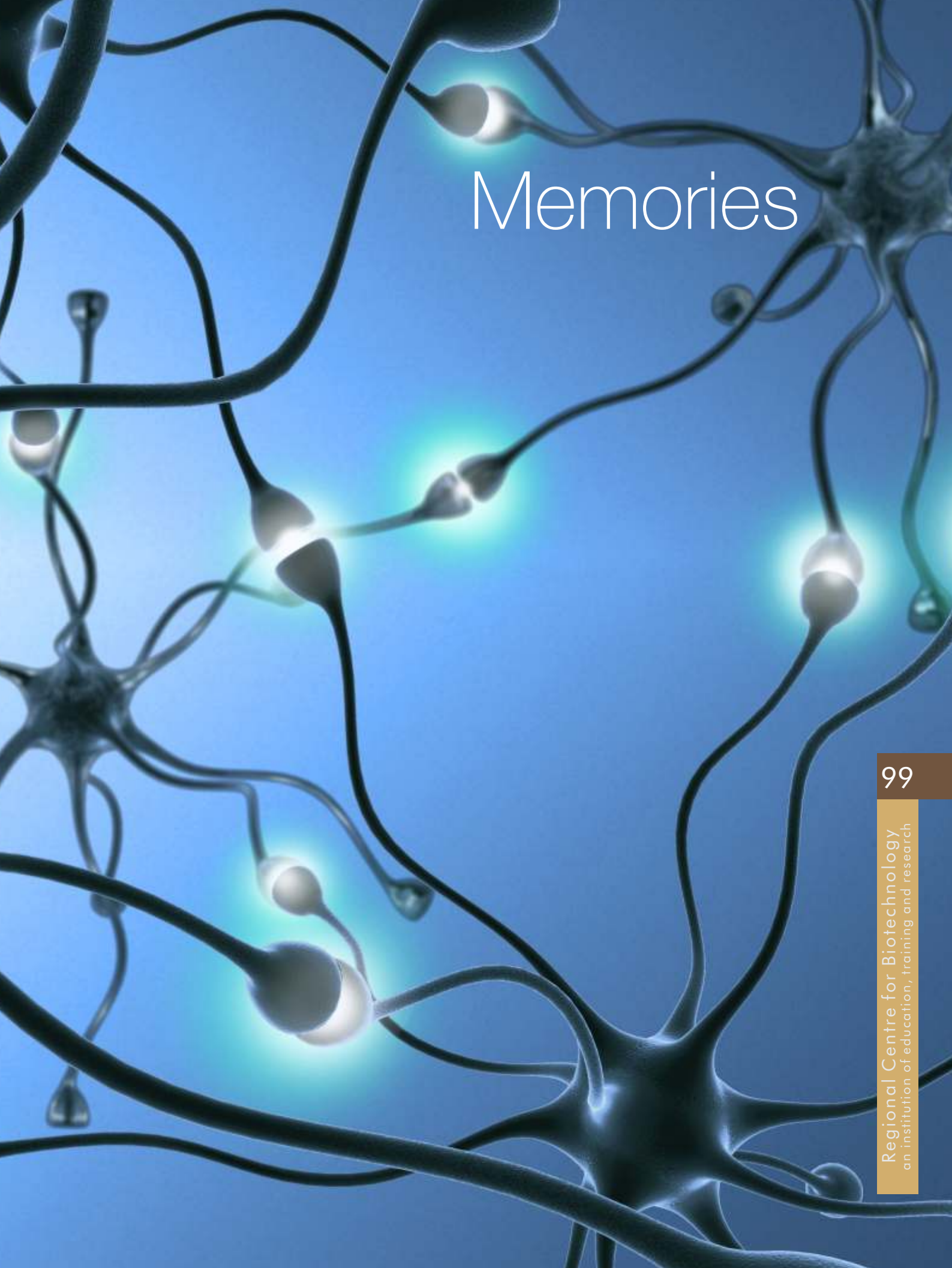
Information Technology

Ms. Alka Chug

NCR-BSC

Sh. Sreeshan Raghvan, Administration
Sh. C. L. Raina, Finance
Sh. Shyam Sunder Budhwar, Engineering
Dr. Ramesh Juyal, Scientific and Technical





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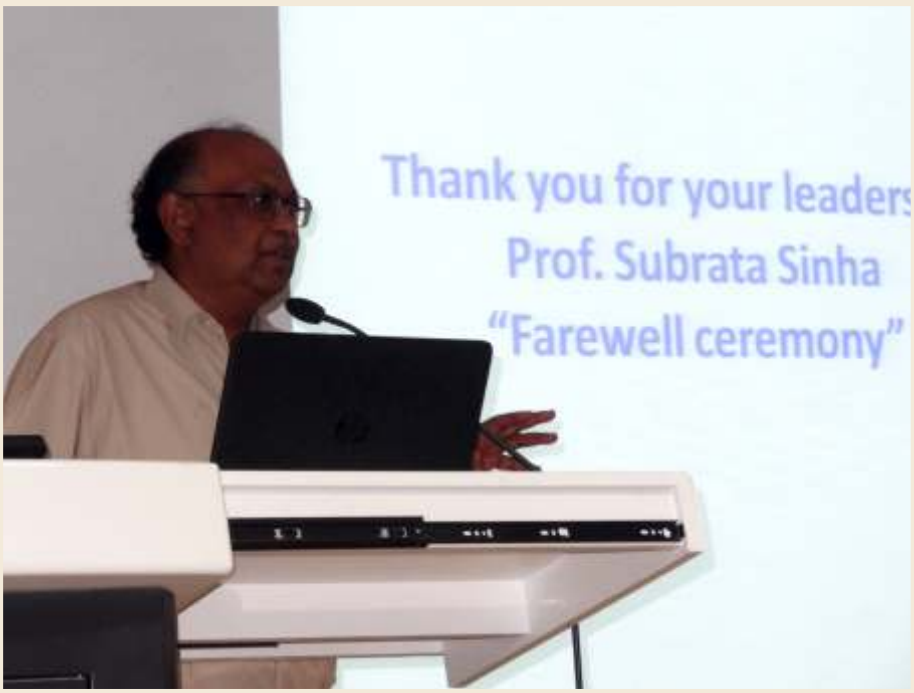
Regional Centre for Biotechnology
an institution of education, training and research

Memories


Welcome
f. Sudhanshu Vrati
Executive Director of RCB


Thank you for your leader
Prof. Subrata Sinha
"Farewell ceremony"


REGIONAL CENTRE FOR BIOTECHNOLOGY


REGIONAL CENTRE FOR BIOTECHNOLOGY


RCB


NCR - BIOTECH SCIENCE CLUSTER
DEPARTMENT OF BIOTECHNOLOGY, GOVT. OF INDIA

UNESCO

United Nations
Educational, Scientific and
Cultural Organization

REGIONAL CENTRE FOR BIOTECHNOLOGY

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