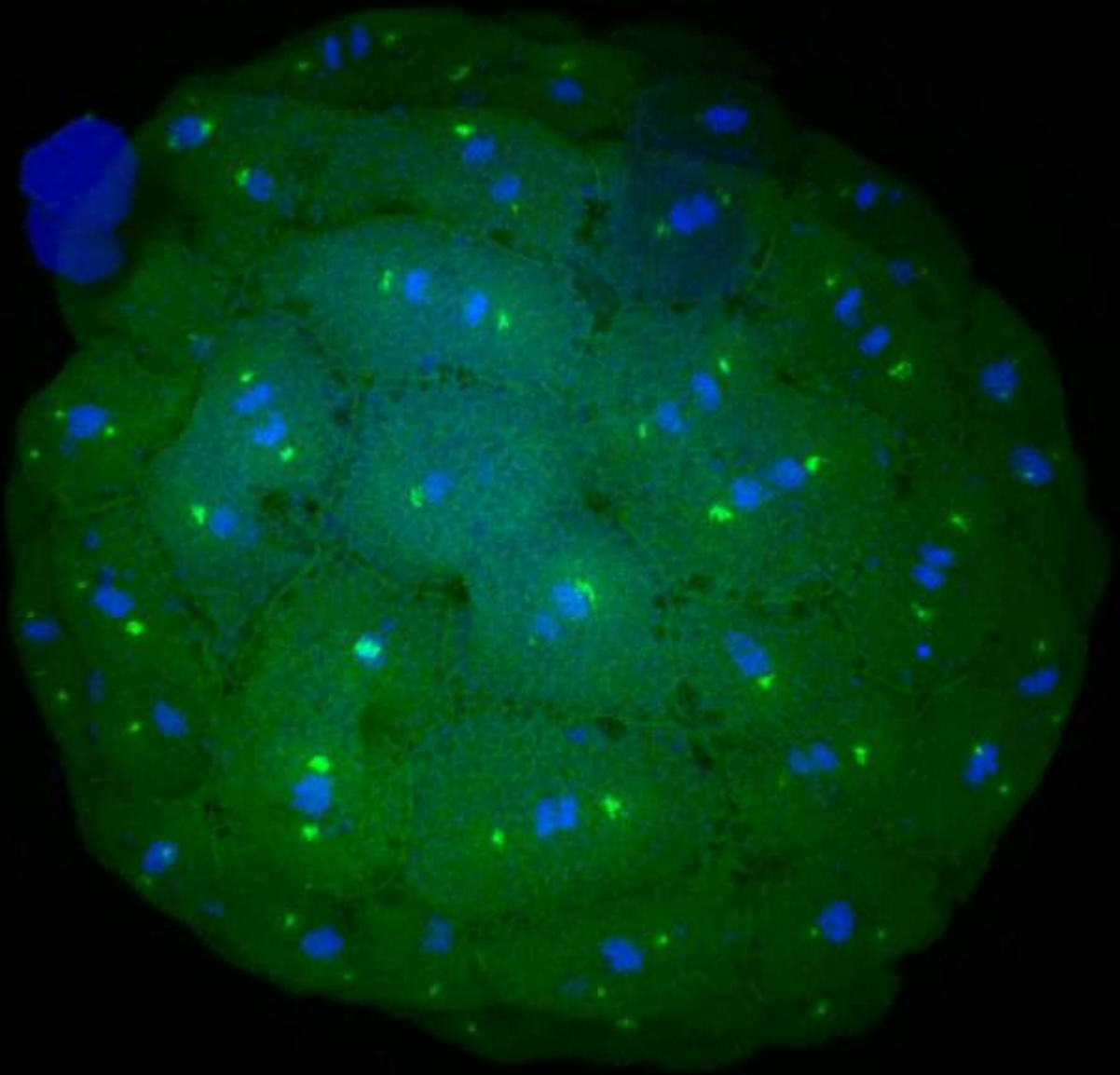
United Nations
Educational, Scientific and
Cultural Organization

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 2014-15



Contents

1. From the Executive Director's Desk 01
2. Mandate of the Centre 03
3. Scientific Reports 05
   - Engineering of Nanomaterials for Biomedical Applications 07
   - Molecular Determinants of Genomic Integrity and Plasticity 13
   - Transcription Regulation: Structure and Mechanism 19
   - Structural Biology of Regulatory Events in Physiological Processes 25
   - Modulation of Host Immunity and Nutrient Allocation by a Biotrophic Pathogen 29
   - Pathophysiology of Procoagulation, Inflammation and Thrombosis in different diseases and Immune Responses 35
   - Effector-triggered Immunity: Elucidating Inositol Regulated Pathways in Assemblies and Signaling by Immune regulators 39
   - Intrinsic Signals that Regulate Skeletal Muscle Structure and Function 43
   - Mechanisms of Cell Division and Cellular Dynamics 47
   - Studies on Biology of Infectious and Idiopathic Inflammation of the Gut 51
   - Post-translational Protein Modifications: Involvement in Cellular processes and Disease regulation 57
   - Structural Biology of Host-Microbial Interactions in Health and Diseases 61
4. Academic Activities & Achievements 65
   - Education & Training 67
   - Workshops & Study Visits 68
   - Lectures Delivered/ Conferences attended/ Visits abroad 71
   - Memberships of Professional/ Academic Bodies/ Editorial Boards 75
   - Distinctions, Honours & Awards 77
   - Publications 78
   - Scientific Colloquium 81
   - Distinguished Lecture Series 82
   - Faculty Mentorship Programme 84
   - Joint UNESCO-DBT Symposium 85
   - Seminars Delivered by Visiting Scientists 86
5. Extramural Activities & Funding 89
   - Multi-institutional Preterm Birth Programme (PTB) 90
   - NCR-Biotech Science Cluster, Faridabad 91
   - Advanced Technology Platform Centre (ATPC) 91
   - Biotechnology Business Incubator 91
   - International Networking 91
   - International and National Responsibilities 93
   - Extramural Grants 94
6. Infrastructure & Development 97
   - Laboratory Infrastructure 98
   - Information Technology Infrastructure 99
7. Financial Information 101
   - Audit Report 103
   - Balance Sheet 104
   - Income and Expenditure Account 105
   - Accounting Notes & Policies 106
8. Institutional Information 109
9. Memories 121

Contents

From the Executive Director's Desk

Last year we had promised to bring out this edition of the Annual Report from our permanent building located on the campus of the NCR Biotech Science Cluster. I am very happy to say that we have been here in Faridabad now for nearly 10 months in our own building located in a beautiful and serene corner of NCR. While relocating to the new campus we, of course, faced usual and novel hurdles. All these barriers could be overcome due to the intense efforts and great cooperation of all my colleagues in different departments of RCB. There were some disruptions in normal operations during the early months of this year but after a short lag period we have been functioning smoothly for the last seven months. The effect of the relocation on scientific productivity was minimized and this is reflected in some excellent publications that have come out in the past few months.

The core research activities are continuously following an ascending trend. The group addressing the role of SUMOylation in Salmonella infection and inflammatory bowel disease (IBD) has uncovered the mechanistic aspects of Salmonella induced alteration in SUMOylation patterns. The laboratory investigating fundamental molecular mechanisms regulating cell division and intercellular communication has highlighted the role of the cytoplasmic dynein subunit Light Intermediate Chain 2 in regulating mitosis and also reported detailed biochemical characterization of a key protein involved in nanoconduit formation. The bio-nanotechnology group has successfully designed ampiphiles targeting important microbial pathogens and these efforts may lead to a new class of antimicrobial drugs.

The laboratory focussed on transcription and gene regulation has obtained deep mechanistic insight regarding an antiactivator important for assembly of flagella in *Pseudomonas aeruginosa*. The research programme addressing pathophysiology of thrombosis in disease conditions have acquired interesting insights regarding hemoglobin (Hb) mediated platelet activation, Hb mediated macrophage activity, Hb mediated stem cell differentiation and dengue virus induced thrombocytopenia. The laboratory interrogating the role of ubiquitin mediated signalling in cellular processes has highlighted the role of Ubiquitin-C terminal hydrolase 1(UCHL1) in regulating Parkinson's disease pathology. The research group investigating intrinsic signals that regulate skeletal muscle development and function has discovered the effect of loss of myosin heavy chain-embryonic on muscle differentiation and mouse development.

The recently initiated programmes in the area of plant biotechnology have progressed well in the past year. The group addressing modulation of plant host immunity and nutrient allocation by an obligate biotroph has optimized a tissue preparation method for infection site-specific expression profiling via laser microdissection microscopy and obtained preliminary evidence for a role for plant SWEET transporters in powdery mildew nutrition. The laboratory that explores signalling pathways in effector triggered immunity of plants

From the Executive Director's Desk

From the Executive Director's Desk

has highlighted the relevance of SUMOylation and the role of inositol-phosphates in regulation and activation of this process.

The group working on Structural Biology of physiological processes has identified genetically distinct antibodies specific to different neutralizing epitopes of influenza virus and this study will soon shed light on the strategies adopted by the immune system to deal with polymorphism within peptide antigens of viral origin. The laboratory investigating the formation of nanoscale protein assemblies that enable bacteria to adhere to biotic and abiotic surfaces has obtained mechanistic insight regarding the assembly of the core of the pilus appendage. The research group focussed on elucidating the mechanisms utilized by molecular determinants of genomic integrity and plasticity to achieve function has shown how a DNA polymerase reduces the sensitivity of *E. coli* towards the nitrofurazone antibiotic.

The academic activities showed great enhancement during the past year. More research scholars, young investigators and post-doctoral fellows joined RCB for training and scientific research. A scheme for engaging Emeritus Scientists has been put in place with a view to enhance the academic and research ambience of the centre. The faculty mentorship program had another successful year and I acknowledge the efforts put in by all mentors towards improving the research conducted at RCB. New episodes of the distinguished lecture series and RCB colloquia were conducted and these events allowed eminent scientists to address young and senior scientists in the national capital region. RCB conducted well-attended international workshops on diabetes and advances in cryoEM and X-ray crystallography and more such interactions will happen in the near future.

The major positive consequence of relocating to the new campus is that the two institutions, RCB and THSTI, have evolved an excellent strategy to share resources. I should acknowledge cooperation from all my colleagues in RCB and THSTI for the exceptional synergy on display. Together, we have reinforced the importance of institutional networking across the NCR Biotech Science Cluster. The enthusiastic support and encouragement from the colleagues in DBT and UNESCO, members of the Board of Governors, the Programme Advisory committee (PAC) and various other statutory committees have also been critical. Now that we are operating in our own campus with most of the infrastructure in place, I am confident that RCB's full potential will be truly realized in the coming years.

Dinakar M. Salunke
Executive Director

Mandate of the Centre

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.

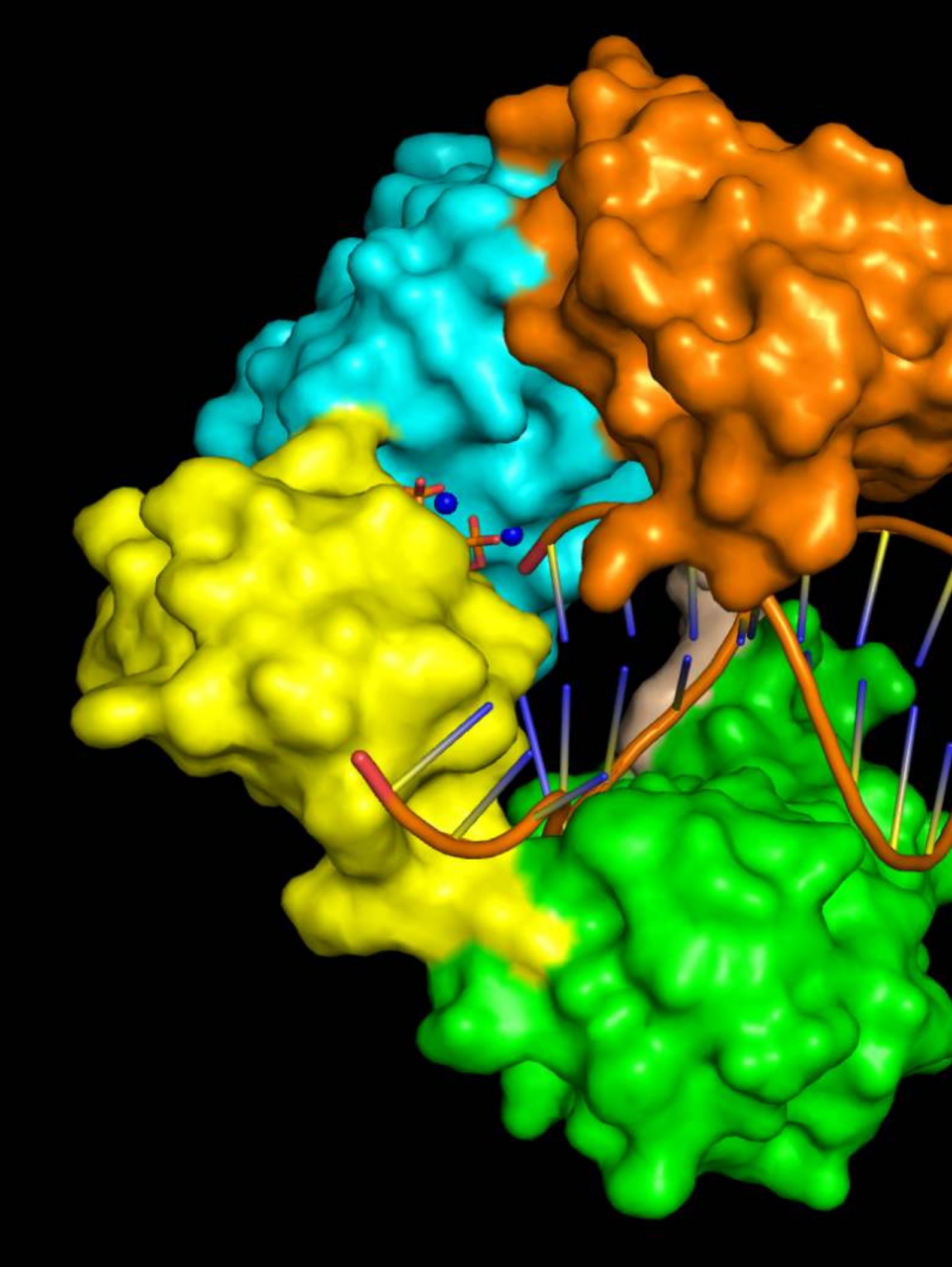
The vision is to produce human resource tailored to drive innovation in biotechnology, particularly in areas of new opportunities and also to fill talent gap in deficient areas. The Centre shall be an institution of international importance for biotechnology education, training and research (and shall, in due course, be constituted as an autonomous body under an Act of the Parliament).

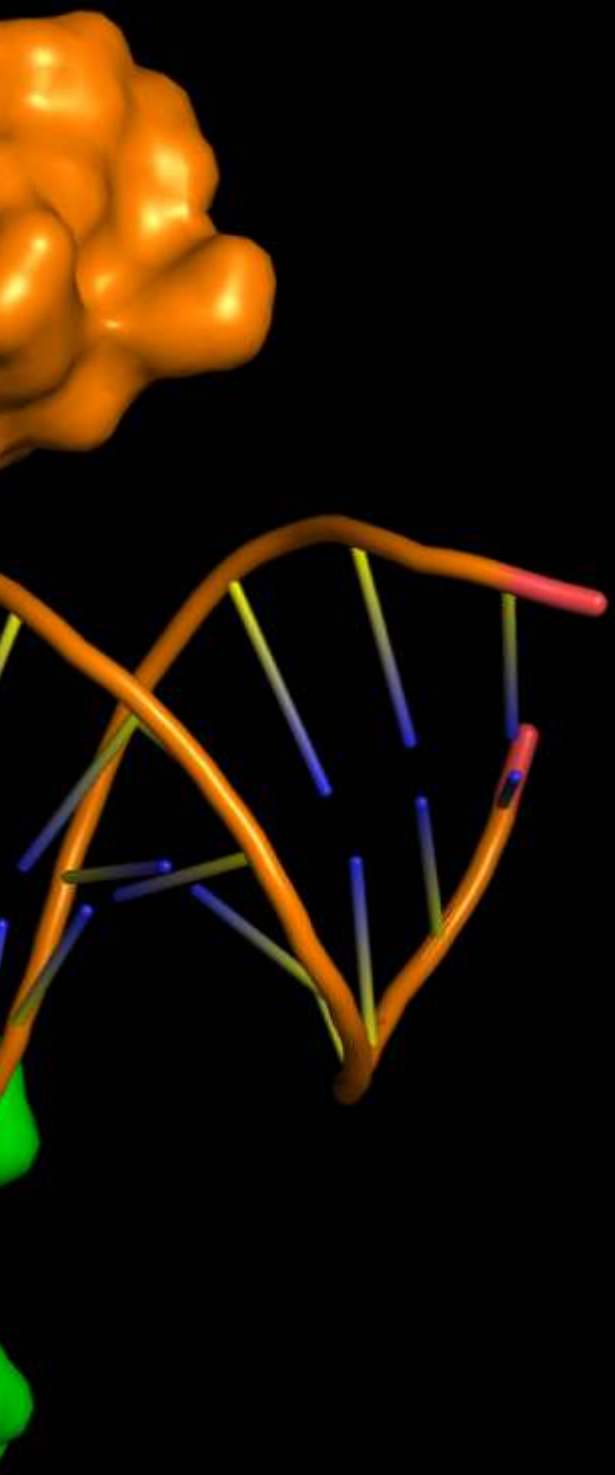
The Centre is regarded as a "Category 2 Centre" in terms of the principles and guidelines for the establishment and functioning of UNESCO Institutes and Centres.

The Centre functions with the following objectives:

- To produce human resource through education and training in a milieu of research and development for application of biotechnology for sustainable development towards building a strong biotech industry through regional and international co- operation with emphasis on novel interdisciplinary education and training programmes, currently not available in the country.
- To develop research programmes of a global quality through international partnerships.
- To establish technology policy development and information dissemination activities.
- To establish desired infrastructure and technology platforms to support above mentioned activities.
- To enable periodic experimentation in design and implementation of biotechnology education and training and to be a source of new concepts and programmes.
- To create a hub of biotechnology expertise in South Asian Association for Regional Cooperation (SAARC) region and more generally in the Asian region to address human resource needs.
- To promote and strengthen South-South & South-North co-operations around issues relevant to biotech education, training, innovation, commercialization and trade; and
- To promote a network of satellite centres in these sub-regions.

Mandate of the Centre





Scientific Reports

Progress during 2014-2015

Annual Report 2014-15

05

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

Engineering of Nanomaterials for Biomedical Applications

Avinash Bajaj

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

Postdoctoral Fellow
Manish Singh
Sandhya Bansal

Ph. D. Students
Vedagopuram Sreekanth
Somanath Kundu
Kavita Yadav
Sandeep Kumar
Nihal Medatwal
Sanjay Pal
Shachi Saluja

Project Staff
Varsha Komala
Rama Sharma

Research Theme

We are using an interdisciplinary approach using synthetic chemistry, cell biology, microbiology, and nanotechnology to address challenges in the area of membrane biophysics, cancer biology and infectious diseases; and to develop nanomaterials for effective therapeutics to cancer and infectious diseases.

Objectives

Objective in the current progress report is to understand the amphiphile-membrane interactions for development of effective strategies for antimicrobial therapy against resistant infections and biofilms.

Progress

Bacterial infections have been associated with serious outbreaks causing alarming situations in many parts of the world. Association of bacterial infections with antibiotic resistance induces life-threatening situations in developing countries like India. Developed countries like Europe and USA do also face the economic and health burden due to development of resistant and persistent infections, as 400,000 multi-drug infections and 25,000 attributable deaths happened in Europe in 2007. Inability to find new drugs since the golden era of 1940-1960s further agitates the problem in antimicrobial therapy.

Currently used antimicrobial therapy involves the use of antibiotics that can target major biosynthetic pathways occurring in bacteria like biosynthesis of DNA, RNA, Proteins, peptidoglycans and folic acid. Presence of bacterial sub-populations having varying antibiotic susceptibility and emergence of multi-drug resistance (MDR) by these bacteria challenges the overall efficacy of antibiotics. Therefore, targeting of these major pathways usually fail in eradicating the multi-drug resistance infections, persistent infections, and biofilms involving slow-growing or non-growing bacteria. Bacterial cell membranes are

crucial to both active and metabolically inactive pathogens and quiescent bacteria, as they need cellular energy and redox homeostasis to maintain cell viability. Therefore, antimicrobial drugs targeting organization of bacterial membrane provide a suitable alternative and promising therapeutic approach for these bacterial infections. Membrane targeting antimicrobials are usually lipophilic in nature that directly interact with bacterial membranes and disrupt their function and its physical integrity. Daptomycin and telavancin disrupt the membrane bilayer and are in clinical use for treating *Staphylococcus aureus* infections.

Natural antimicrobial peptides (AMPs) play a major role in innate immunity in lowering the bacterial and viral infections. Engineered AMPs mimics face challenges of their high cost, proteolysis degradation and poor selectivity for a particular bacterium. Irreversible drug-membrane interactions are crucial for effective binding of drugs to membrane, its penetration through lipid bilayer of cell membranes, its bioactivity, and intracellular actions that dictate intracellular fate of the drug. Amphiphiles are lipid like molecules with charged head groups and hydrophobic parts. Amphiphiles have the tendency to interact with cell membranes of different cell types and causing cellular toxicities. Due to multiple target sites of membrane disruptive agents, it will be difficult for bacteria to acquire resistance against these agents. Therefore, membrane-active agents should have the properties of a) bacterial membrane targeting b) ability to kill target pathogens selectivity c) disrupt multiple parts of bacteria for effective antibacterial action and low resistance. The synthetic chemicals or natural products targeting bacterial membranes would face the challenge of disrupting the mammalian membrane, and lack of optimization to attain pathogen selectivity.

Bile acids due to their inherent facial amphiphilic character can easily mimic AMPs that possess cationic charged surface on one side and hydrophobic domain on other side. Due to their charged surfaces, these AMP's have the potency to interact with cell membranes of bacteria and cause cellular toxicity. These amphiphile-cell membrane interactions usually involve the electrostatic interactions between charged amphiphiles and negatively charged phospholipids/glycans of cell membranes; followed by integration of the amphiphiles in the membranes causing disruptions in cell membrane. Bile acids are major constituents of bile derived from cholesterol that help in absorption of fats and fat-soluble vitamins. Bile acids present interesting materials for biomedical applications due to a) their facial amphiphilic, b) high biocompatibility, c) existence of diverse chemical scaffolds, and d) presence of reactive functional groups. Therefore, engineering and interactions of bile acid based materials with biomembranes would be critical for design of new nanomaterials for biomedical applications.

We synthesized a library of amphiphiles where variable head group from soft-charged ammonium (AMM) to hard-charged trimethyl ammonium (TMA), N-methyl piperidine (PIP), pyridine (PYR), and dimethylamino pyridine (DMAP) were conjugated to four bile acids, lithocholic acid (LCA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), and cholic acid (CA). Antibacterial activities of amphiphiles against mycobacterial species, *M. smegmatis* (Msm), *M. tuberculosis* H37Rv (Mtb) and *M. bovis* BCG showed that DMAP derived amphiphiles were most effective against Mtb inhibiting mycobacterial growth in range of 0.78-6.25 μM; whereas ammonium head group derived soft-charged amphiphiles possess no activity against mycobacteria. Antibacterial activities against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria suggested that DMAP derived hard-charged multiple headed amphiphiles are less potent than soft-charged amphiphiles CA-AMM3 amphiphile that are highly specific in its ability to inhibit growth of *E. coli*/*S. aureus*. Among the series single headed LCA based amphiphiles were not active against neither of the bacteria.

Cytotoxicity studies of amphiphiles against mammalian cells showed that single-charged are most cytotoxic, whereas hard-charged multi-headed amphiphiles are least cytotoxic. Hemolytic studies against showed high therapeutic index (MHC50/MIC99) of multi-headed CA based amphiphiles. Mechanistic studies of the amphiphiles suggested the dose-dependent bactericidal killing of *E. coli/S. aureus* by CA-AMM3 and of Msm with CA-DMAP3. Membrane perturbation studies suggested the disruption of *E.coli/S. aureus* membranes with CA-AMM3 and proton motive force got disturbed by CA-DMAP3 that were further confirmed by AFM studies. EDTA is known to destabilize the lipopolysaccharides and proteoglycan layer of the bacteria. Therefore there was 16-64 fold increase in activity of CA-DMAP3 against *E. coli* and *S. aureus* bacteria whereas activity of CA-AMM3 got enhanced by 2 fold only suggesting that presence of LPS and proteoglycan layers does not allow the bactericidal action of CA-DMAP3 against E. coli/S. aureus.

To unravel the mechanism of differential activity of amphiphiles against different bacterial species, we studied the interactions of these amphiphiles with model bacterial membranes, and observed the change in membrane hydration and fluidity of model membranes on interactions with these amphiphiles. Membrane surface hydration studies suggested that mycobacterial model membranes (PE:PG:TDM) are drier as compared to *E. coli* and *S. aureus* based model membranes (PE:PG:LPS and PE:PG). Incubation of CA-AMM3 causes increased dehydration of *E. coli* and *S. aureus* based model membranes due to electrostatic interaction thereby responsible for its antibacterial action, and minor dehydration was observed on incubation with model mycobacterial membranes (PE:PG:TDM). CA-DMAP3 on the other hand did not induce much dehydration in either of the model membranes, due to presence of hydrophobic head groups. We then studied the influence of CA-AMM3 and CA-DMAP3 on membrane fluidity of model membranes. CA-AMM3 did not induce ay change in membrane fluidity due to presence of strong electrostatic interactions with charged head groups and poor interactions with hydrophobic tails. Incidentally incubation of mycobacterial membranes with CA-DMAP3 enhanced their fluidity that might be due to strong hydrophobic interactions.

To study the effect of side-chain hydrophobicity, we synthesized derivatives of CA-AMM3 amphiphile where we varied the methyl ester of cholic acid with different hydrophobic long chains like methyl, ethyl, butyl, hexyl, octyl, decyl and dodecyl. Antibacterial activities of CA-AMM3 amphiphiles suggested very interesting structure-activity relationship, where activity of the amphiphiles increases with increase in length of the side-chain hydrophobic tail up to hexyl long chain, and then decreases with further increase in long chain. We observed that CA-AMM3 with hexyl ester is most effective amphiphile against *E.coli/S. aureus*. Antibacterial activities of these amphiphiles against five other laboratory strains also suggested the same structure-activity relationship. Membrane permeabilization studies confirmed the bactericidal effect and membrane perturbations of bacteria using hexyl-derived amphiphile. Cytotoxicity studies against mammalian cell type and hemolytic activities showed that introduction of hexyl group enhances the activity of the hexyl-derived amphiphiles against these cell types as compared to methyl ester analogue, although these activities were observed at higher concentrations than their bactericidal effect. To overcome these challenges, we engineered ~120 nm nanoparticles from hexyl-derived amphiphiles with the help of PEG derived cholesterol. *In vitro* assays showed the potent antibacterial effect of these nanoparticles against lab bacterial strains. We are planning to explore the *in vivo* potential of these particles along with pharmacokinetic studies.

Scientific Reports

Future plans

In spite of many encouraging studies on membrane-active agents, antimicrobial therapy using membrane active agents faces many challenges a) polymicrobial composition of medical biofilms, and, b) membrane active antimicrobials that also target Gram-negative biofilms, c) commonly associated toxicity like nephrotoxicity, d) poor pharmacokinetics as these lipophilic molecules have tendency to bind to proteins and may not distribute well in to all tissues, and e) urgent need for antibiotics that decrease the time required to effectively treat biofilm infections and other persistent infections. Therefore, for targeting these challenges; there is need for effective anti-biofim strategy to eliminate multiple bacterial species, and synergize with other antibiotics to target multi-drug resistance and persistence infections.

In future, we plan to synthesize a) Conjugates of existing antibiotics with cell penetrating amphiphiles for enhanced permeability, b) library of facial amphiphiles with different head groups to modulate their interactions with bacterial membrane to target MDR infections, c) facial amphiphile-drug conjugates with existing anti-infectious drugs to unravel the combined effect of facial amphiphiles and existing drugs. These amphiphiles will be studied for their anti-bacterial activity against different bacterial species. We would then study the activity of these amphiphiles and amphiphile-drug conjugates against different mammalian cell types and red blood cells to explore the selectivity of these amphiphiles. Antibacterial potential of these amphiphiles will then be explored against clinical isolates of different bacterial species, biofilms, and different MDR bacterial species and compared with commonly used antibiotics. We would explore the ability of the bacteria to generate drug resistance towards these engineered amphiphiles. Further, nanoparticle therapeutics from these amphiphiles can improve the patient compliance by reducing the frequency of drugs. These amphiphiles and their nanoformulations will then be explored for pharmacokinetic and bio-distribution profiles, and tested in different *in vivo* infectious models.

Publications

1. Yadav K, Bhargava P, Bansal S, Singh M, Sreekanth V and Bajaj A (2015) Nature of the charged head group dictates the anticancer potential of lithocholic acid-tamoxifen conjugates for breast cancer therapy. ***Med Chem Commun*** 6:778.
2. Kumar S, Bhargava P, Sreekanth V and Bajaj A (2015) Design, synthesis, and physico-chemical interactions of bile acid derived dimeric phospholipid amphiphiles with model membranes. ***J Colloid Interface Sci*** 448:398.
3. Kundu S, Kumar S and Bajaj A (2015) Cross-talk between bile acids and gastrointestinal tract for progression and development of cancer and its therapeutic implications. ***IUBMB Life*** 67:514.
4. Sharma A, Pohane A, Bansal S, Bajaj A, Jain V and Srivastava A (2015) Cell penetrating synthetic antimicrobial peptides (SAMPs) exhibiting potent and selective killing of mycobacterium by targeting its DNA. ***Chem Eur J*** 21:3540.
5. Singh M, Bansal S, Kundu S, Bhargava P, Singh A, Motiani R. K, Shyam R, Sreekanth V. Sengupta, S and Bajaj A (2015) Synthesis, structure-activity relationship, and mechanistic investigation of lithocholic acid amphiphiles for colon cancer therapy. ***Med Chem Commun*** 6: 192.

6. Bansal S, Singh M, Kidwai S, Bhargava P, Singh A, Sreekanth V, Singh R andBajaj A (2014) Bile acid amphiphiles with tunable head groups as highly selective non-hemolytic antitubercular agents. ***Med Chem Commun*** 5: 1761.

7. Singh K, Verma V, Yadav K, Sreekanth V, Kumar D, Bajaj A and Kumar V (2014) Design, regioselective synthesis and cytotoxic evaluation of 2-aminoimidazole-quinoline hybrids against cancer and primary endothelial cell. ***Eur J Med Chem*** 87: 150.

8. Singh M, Kundu S, Reddy A, Sreekanth V, Motiani RK, Sengupta S Srivastava A and Bajaj A (2014) Injectable small molecule hydrogel for localized and sustained in vivo delivery of Doxorubicin. ***Nanoscale*** 6:12849.

Scientific Reports

Molecular Determinants of Genomic Integrity and Plasticity

Deepak T. Nair

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

Young Investigator
Vaibhav K. Pandya

Ph. D. Students
Rahul Sharma
Jithesh Kottur
Shivlee Nirwal
Naveen Narayanan
Deepankar Singh
Manhar S. Rawat
Mary K. Johnson
Shilpi Nagpal
Minakshi Sharma

Research Theme

We study molecules that either maintain genetic integrity or render genomic plasticity. Our efforts will provide mechanistic insight into how organisms evolve and adapt to the environment.

Objectives

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 probably ensures 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 my laboratory are (a) Translesion DNA synthesis (b) Stress-Induced Mutagenesis (c) DNA Mismatch repair (d) Stress-induced epigenetic modification (e) Regulation of Genomic recombination (f) Transposition and (g) 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.

Scientific Reports

Progress

1. Translesion DNA Synthesis

The action of genotoxic agents leads to the presence of damaged nucleotides –termed as lesions- in the genome. These lesions inhibit replicative dPols and thus stall the replication fork. Many organisms possess low-fidelity dPols that serve to rescue genome replication stalled at damaged nucleotides. We aim to unearth the structural and chemical strategies used by prokaryotic Y-family dPols to achieve translesion DNA synthesis. Towards this end, we are carrying out a rigorous biochemical and structural analysis of DNA Polymerase IV (PolIV) - a representative member of this class of dPols from *Escherichia coli*.

a. Translesion DNA synthesis to rescue genome replication stalled at minor groove adducts

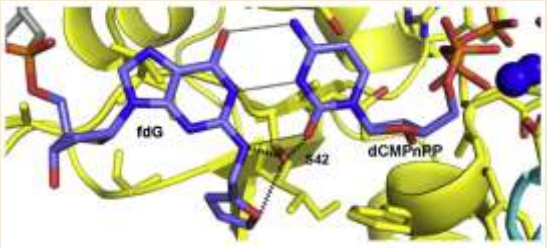


Figure 1. Close up of the active site of the PolIV$_{fdG:dCMPnPP}$ complex. The fdG adduct is present at templating position and is paired to the incoming nucleotide. The S42 residue forms interactions with the damaged template and incoming nucleotide.

A number of genotoxic chemicals react with the N2- atom of deoxyguanosine resulting in the appearance of minor groove adducts of this nucleotide- a majority of which can block replication. The N2-furfuryl adduct of guanine (fdG) arises due to the action of the antibiotic nitrofurazone and serves as a model N2- deoxyguanosine adduct. PolIV bypasses this adduct with high accuracy and this property considerably reduces the sensitivity of E. coli to nitrofurazone (NFZ).

Steady-state kinetic analysis showed that PolIV can incorporate dCTP opposite the fdG nucleotide with higher catalytic efficiency than undamaged dG. We have determined three structures of PolIV in complex with DNA containing N2-furfuryl adduct at the templating and two different downstream positions [Kottur et al. (2015) Structure 23:56]. These structures provide snapshots of the enzyme in the incorporation (Fig. 1) and extension modes during translesion bypass of the fdG adduct. The three structures show that the relative orientation of different domains of PolIV allows formation of an extended cavity that is occupied by the furfuryl group during incorporation as well as extension modes. The structures- along with allied biochemical and functional data- allow formulation of a simple and elegant mechanism for the observed accurate translesion synthesis by PolIV past the replication-blocking N2-deoxyguanine adducts with high catalytic efficiency. Overall, this study provides the mechanism utilized by PolIV to neutralize the antimicrobial activity of NFZ and thus improve the viability of *E. coli* in the presence of this nitrofuran antibiotic.

At present, we are engaged in probing the ability of PolIV to bypass N2-adducts of varying complexity. These adducts appear in the genome due to the action of different genotoxic agents.

b. PolIV and oxidative lesions

Reactive oxygen species (ROS), such as hydroxyl radicals can react with DNA and lead to the formation of DNA lesions. The most common lesion induced by ROS is the 8-oxodeoxyGuanosine (8oxoG) lesion. The presence of this lesion in the genome results in G:C to T:A transversions. These mutations appear because 8oxoG adopts the syn conformation that can form a Hoogsteen base pair with A and replicative dPols tend to incorporate an A opposite 8-oxoG. Steady-state kinetic experiments showed that PolIV can preferentially incorporate the C nucleotide opposite this lesion. Using a combination of macromolecular crystallography and steady state kinetic analysis of mutant and native enzyme, we have discovered the structural mechanism utilized by PolIV to mediate accurate bypass of this otherwise mutagenic lesion.

2. Stress-Induced Mutagenesis

Low fidelity DNA polymerases, such as PolIV have been shown to participate in stress-induced mutagenesis. The expression of these enzymes is up-regulated when *E. coli* encounters environmental and nutrient stress and error-prone DNA synthesis by PolIV 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 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.

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 also known to promote frameshift mutations and at present, efforts are underway to understand the structural basis of this activity of PolIV.

3. 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, we aim 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.

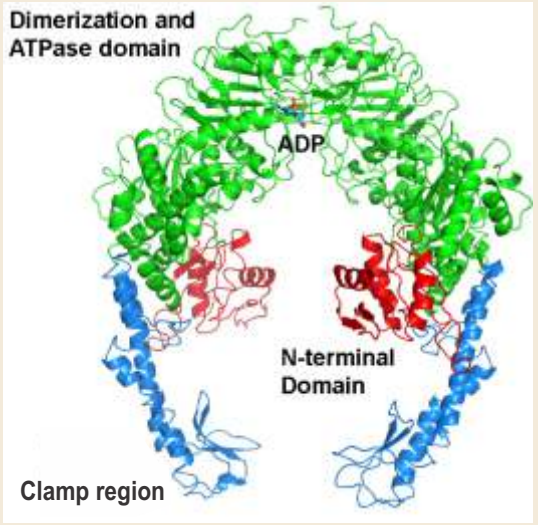


Figure 2. Structure of the MutS homolog from Neisseria at 2.65 Å

The functional form of NgoS and orthologs is an asymmetric dimer. Two monomers associate to form an oval disc with a tunnel towards the bottom into which DNA is loaded to scan for mismatches. In the DNA-bound form, this tunnel of NgoS will be closed from all sides and therefore it is unclear how DNA is threaded through this cavity. The structure of NgoS in its apo- state shows that, in the absence of DNA, the clamp region of the two monomers do not contact each other (Fig. 2). In addition, the N-terminal domain (NTD) is oriented away from each other resulting in the widening of the DNA channel. As a result, an opening is created between the clamp regions that will allow access to the DNA tunnel. DNA can enter the tunnel

through this opening. Following this, the clamp regions and the NTDs reorient to ensure that DNA is threaded through the correct tunnel. The association of MutS with DNA should, therefore, result in a lowering of the hydrodynamic radius and this was confirmed by Dynamic Light Scattering studies. Overall, the structure of NgoS in the absence of DNA and allied biophysical studies reveal how a functional MutS-DNA complex is assembled.

4. Epigenetic modification to respond to stress in the environment

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 deal with stress in the environment. We aim to elucidate the regulatory mechanisms that permit these enzymes to act only under specific environmental conditions.

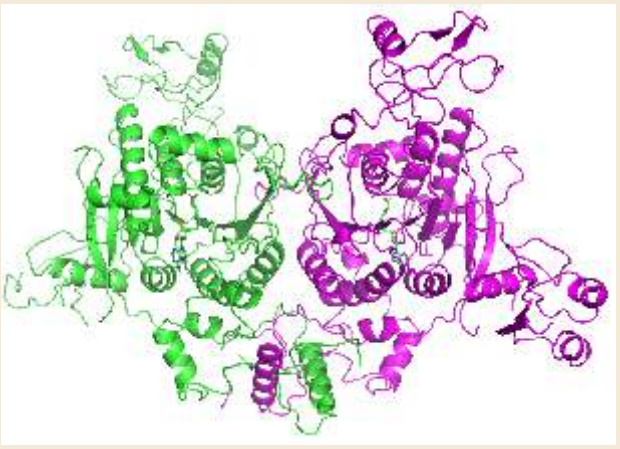


Figure 3. Structure of HP0593:SFG complex at 2.75 Å

The expression of the HP0593 dMtase is upregulated when the pathogen encounters low pH. HP0593 is a Type III 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). The structure (Fig. 3) coupled with biochemical and biophysical analysis of site-specific mutants of HP0593 suggests that a functional dimer 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.

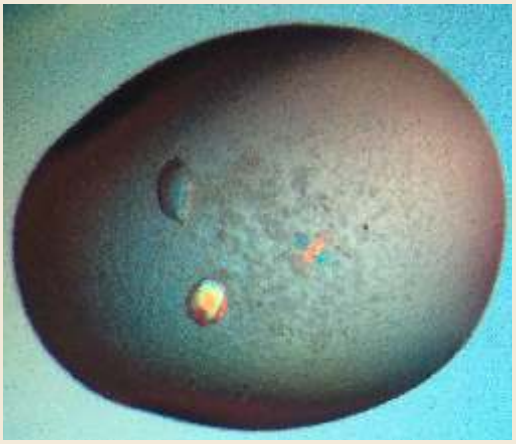


Figure 4. Crystals of ΔSMR domain of HpMutS2

5. Regulation of Genomic Recombination

Homologous recombination (HR) is responsible for the integration of exogenous DNA and rearrangements in the genome. The variation generated due to HR enables prokaryotes to survive in a dynamic environment. HR has to be regulated so that genomic rearrangements do not occur at a frequency that can compromise genetic viability. This is especially true in the case of the pathogen *Helicobacter pylori*, as it is naturally

Scientific Reports

competent with an enhanced ability to uptake and integrate foreign DNA. MutS2, from *H. pylori*, has been shown to suppress HR and thus, serves to optimally calibrate the frequency of genomic rearrangements. We aim to unearth the structural mechanism utilized by HpMutS2 to inhibit recombination and towards this end have purified the protein to high homogeneity. We have recently obtained crystals for the module- named as ΔSMR- that recognizes recombination intermediates.

6. Methods in X-ray Crystallography

We are also involved in improving methods of structure determination. In the past year, we participated in a study to develop a protocol for rapid structure determination using the weak anomalous signal from sulphur atoms in proteins. The method involves collection of highly redundant datasets through exposure of the crystal at different orientations to an X-ray dose that does not damage the crystal [Weinert et. al. (2015) Nature Methods 12:131]. Such a protocol enabled rapid structure determination from two different crystals provided by our laboratory, including that of a protein-DNA complex.

Future Plans

Using biochemical, structural tools coupled with functional assays, we aim to shed light on the mechanism utilized by different molecular determinants of integrity and plasticity to achieve function. The information derived from these studies will be utilized to design further experiments that will provide deeper insight into the role of these molecules in sustaining life of the corresponding organism.

Genomic plasticity allows pathogens to develop resistance against therapeutic and prophylactic agents. Perturbation of function of molecules that modify the genome will considerably potentiate the effect of available drugs and vaccines. The mechanistic insight that we derive from studies on molecular determinants of genomic plasticity will be used to discover chemical inhibitors that can serve as lead molecules for discovery of novel therapeutic agents

Publications

1. Nair DT, Kottur J and Sharma R (2015) A rescue act: Translesion DNA synthesis past N2-deoxyguanosine adducts. ***IUBMB Life*** 67:564.
2. Kottur J, Sharma A, Gore KR, Narayanan N, Samanta B, Pradeepkumar PI and Nair DT (2015) Unique Structural Features in DNA Polymerase IV enable efficient bypass of the N^2-Adduct induced by the Nitrofurazone antibiotic. ***Structure*** 23:56.
3. Weinert T, Olieric V, Waltersperger S, Panepucci E, Chen L, Zhang H, Zhou D, Rose J, Ebihara A, Kuramitsu S, Li D, Howe N, Schnapp G, Pautsch A, Bargsten K, Prota AE, Surana P, Kottur J, Nair DT, Basilico F, Cecatiello V, Pasqualato S, Boland A, Weichenrieder O, Wang BC, Steinmetz MO, Caffrey M and Wang M (2015) Fast native-SAD phasing for routine macromolecular structure determination. ***Nature Methods*** 12:131.

Scientific Reports

Transcription Regulation: Structure and Mechanism

Deepti Jain

Young Investigator
Amit Yadav

Postdoctoral Fellow
Mrittika Sengupta

Ph. D. Students
Chanchal
Priyajit Banerjee
Anuj

Project Staff
Anuska Das

Research Theme

Using an integrated approach, involving structural tools, biophysical techniques, biochemical methods and functional assays our laboratory investigates the molecular mechanism of transcription regulation in different physiological contexts.

Objectives

We aim to elucidate the molecular mechanism of different stages of transcription regulation and mRNA formation. Towards this end, we have focussed on elucidating

- The mechanism utilized by bacterial enhancer binding proteins and their associated factors to regulated transcription
- The mechanism utilized by single subunit mitochondrial RNA polymerase for initiation of synthesis and elongation of the mRNA transcripts
- The allosteric mechanism that renders transcription factors responsive towards small metabolites
- The mechanism of stress-induced crosstalk between two component systems

Progress

1. Structural mechanism utilized by bacterial Enhancer Binding Proteins (bEBPs) to regulate transcription initiation

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. These proteins contain several conserved motifs such as the walker A, walker B motifs, arginine fingers etc. In addition they interact with 54-RNAP to activate transcription. 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. A central domain that interacts with the 54 is responsible for transcription activation and ATP hydrolysis. Although structures of various domains of 54-dependent activators and CryoEM reconstruction of one of the activator bound to RNAP are 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.

Scientific Reports

In order to answer these questions, we have employed FleQ, a master regulator of flagellar genes in *Pseudomonas aeruginosa (Psa)* as a model system. FleQ controls the expression of flagellar genes in a 54 dependent fashion and is present at the apex of the flagellar transcription cascade. Deletion or mutation of fleQ gene makes the bacterium non-motile. We have cloned expressed and purified full length FleQ as well as its various domains to high homogeneity. Crystallization trials of constructs with different domains and domain combinations alone or in complex with ATP or DNA are currently ongoing. The N-terminus domain or the receiver domain of FleQ in its apo- state has been crystallized. Diffraction data have been collected upto 2.2 Å resolution at the BM14 beamline at ESRF (European Synchrotron Radiation Facility). Selenomethionine labelled protein has been prepared and efforts to obtain single crystals are underway. The structure will shed light on the oligomeric status of FleQ-NTD and mechanism of activation of FleQ.

The activity of FleQ is in turn regulated by a protein called FleN. FleN is a putative ATP/GTP binding protein that interacts directly with FleQ without affecting its DNA binding ability. Mutation of *flen* results in a multiflagellated bacterium which shows chemotactic defects due to upregulation of flagellar genes. 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, we are studying the molecular interactions between FleQ and FleN. To begin with, we have determined the crystal structure of FleN in complex with non-hydrolysable analog of ATP (AMPPNP) at 1.66 Å resolution. The Apo-FleN failed to crystallize in the same condition as the FleN-AMPPNP complex indicating that the dimeric assembly is present only in the presence of ATP analog. Crystals of apo-FleN were obtained in non-overlapping conditions and the structure of Apo-FleN was determined using SAD experiment with the data collected at the beamline BM14, ESRF at 1.55 Å resolution. The Apo protein crystallized as a monomer and is significantly different from the ATP-bound form. It is clear from the comparison of the two structures that the protein undergoes drastic conformational changes on dimerization (Figure 1). On the basis of the crystal structure, we have identified constructed and purified FleN mutants.The ability of wildtype and mutant FleN to inhibit ATP hydrolysis by FleQ was assayed. It was found that the wildtype FleN was able to inhibit the ATPase activity of FleQ. However, the mutant FleN, failed to do so. Currently we are in the process of preparing the knockout of these proteins in *Psa* so that the structural studies can be validated *in vivo*.

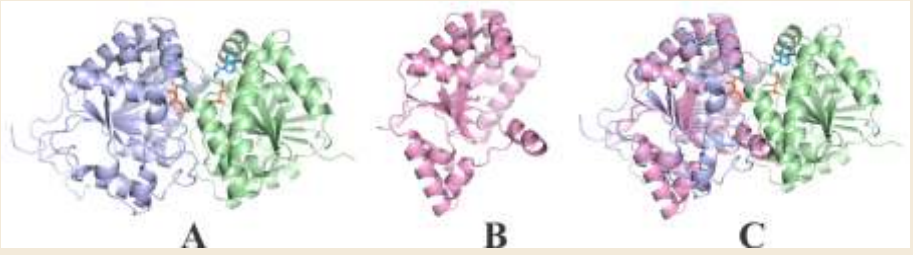


Figure 1: (A) Crystal Structure of the antiactivator-AMPPnPcomplex. (B) Crystal Structure of the monomeric antiactivator. (C) Superimposition of the monomer A from the dimeric and apo structures

Scientific Reports

2. Biochemical and structural studies on the single polypeptide mitochondrial RNA polymerase – RpoTm

Single subunit RNA polymerases 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.

Figure 2: Domain organization of RpoTm

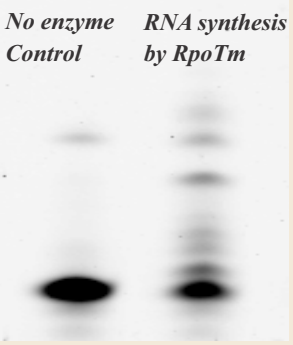


Figure 3: In vitro transcription by RpoTm

AtRPoTm has two domains the N terminal domain and a C terminal domain (Figure 2). C terminal domain of AtRPoTm is a well conserved domain having characteristic right handed topology for polymerases. It has thumb, palm and finger. In the palm region there are two well conserved aspartic acid residues (D677 and D909) which are responsible for the catalytic activity of the protein. In the C terminal domain there is also a specificity loop involved in the promoter recognition. We have cloned expressed and purified the enzyme in large quantities and have demonstrated that the purified enzyme is capable of performing transcription *in vitro* (Figure 3). Crystallization experiments are underway for apo enzyme and initiation complex.

3. Allosteric mechanism utilized by transcription factors responsive towards small metabolites

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 [Jain D. (2015) IUBMB Life 67:556]. AraR protein is the key regulatory protein of the L-arabinose metabolism in *Bacillus subtilis*. 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

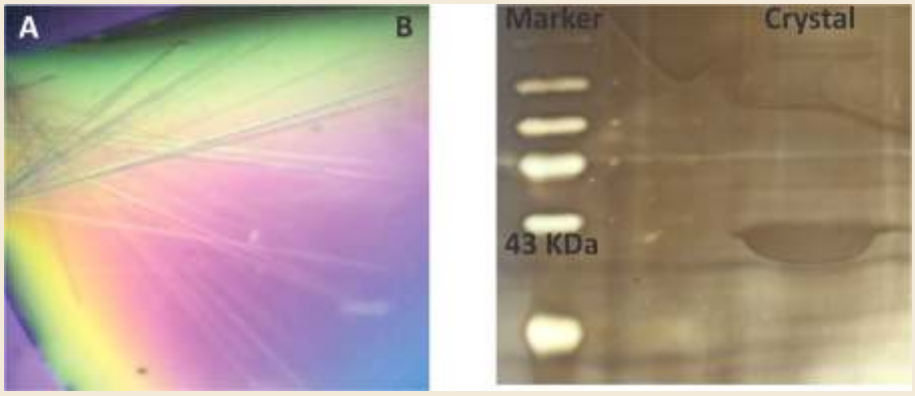


Figure 4: (A) Crystal of full length AraR-L-arabinose complex. (B) The silver stained SDS-PAGE gel shows that the crystals are of full length protein

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 DNA are known. We have recently crystallized the full length AraR in complex with L-arabinose. X-ray diffraction data was collected at 3.2 Å resolution. The crystals belong to space group P21. 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.

AraR binds to eight different operator sequences governing five different promoters and has two different modes of transcriptional repression. We have crystallized AraR-DNA binding domain in complex with four different natural operators. There structures shed light on the plasticity of transcription factors which endows them with the ability to tolerate differences in operator DNA sequences. This observation is in line with recent studies probing specificity of transcription using CHIP-SEQ and also sheds light on evolution of transcription factors.

4. Structure-function studies of the crosstalk between the two-component circuits in *Staphylococcus aureus (Sta)*

The two component systems are signalling 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 *Sta*, 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. We will carry out a rigorous

structural investigation of the interactions responsible for cross talk between the VraSR and GraSR regulon. We will investigate the role of GraX in communicating the signals for cross talk between VraSR and GraSR regulon. We have cloned the graX, graS, vraG, vraR, and vraS genes into expression vectors. Attempts are underway to express and purify N-terminus GST tagged GraX. The purified protein will be subjected to crystallization trials. We will also structurally characterize these interactions by co-crystallizing the relevant proteins in order to understand the mechanism of the "Cross-talk" between the two two-component systems. The ΔgraX knockout mutant strain of *Sta* is being prepared. The effect of this mutation will be analysed using qRT-PCR with genes under both VraSR and GraSR regulons. Antibiotic resistance in *Sta* is 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*.

Future plans

In the future, we will begin investigating the interactions between bacterial EBPs and 54-RNAP. Initially, the pull down and bacterial two hybrid experiments will be carried out to map the interacting interface. The complexes of regulatory factors with different RNAP subunits and cognate DNA sequences will be determined by macromolecular crystallography. In addition, we aim to develop methods that will allow validation of structural mechanisms derived in the appropriate organism. We will design and employ *in vivo* assays that in conjunction with aforementioned structural and biochemical tools- will provide a deeper and more fundamental understanding of the molecular basis of gene regulation.

Publications

1. Jain D (2015) Allosteric control of transcription in GntR family of transcription regulators: A structural overview. ***IUBMB Life*** 67:556.

Structural Biology of Regulatory Events in Physiological Processes

Dinakar M. Salunke

Young Investigator
Sunil K. Tripathi

Postdoctoral Fellow
Ashish Kumar

Ph. D. Students
Sharad Vashisth
Abha Jain
Harmeet Kaur
Sarita C. Sharma
Sheenam Verma
Meha Shikhi
Zaid K. Madani

Project Staff
Bahadur Singh Gurjar

Research Theme

Understanding the physico-chemical principles and mechanistic details of physiological processes associated with immune response, allergy and host-pathogen interaction is the theme of this programme.

Objectives

- Structural proteomics of food allergens
- Analysis of the structural principles of immune recognition in the context of antibody pluripotency
- Structural and molecular bases of host-pathogen interactions

Progress

Immune system is known to be highly specific and can distinguish the subtle differences in the antigens. However, degeneracy in immune recognition is often observed. While, the degeneracy to similar or related antigen is expected, it seems to extend to chemically distinct antigens as well. Cross-reactivity as seen in both germline and affinity-matured antibodies is in defiance to the 'rule of specificity'. We have extensively analyzed in the past the degeneracy of antigen recognition by polyclonal as well as monoclonal antibodies [Kaur & Salunke (2015) IUBMB Life 67:498]. It has been proposed that the affinity matured antibodies are evolved in such a way that their shape and charge complimentarity towards the epitope may increase and therefore, resulting in the reduction of inherent flexibility. Our studies demonstrate that the immune system is not only capable of generating mAbs with narrow specificities but also those which possess different mechanisms to counter subtle differences between antigens. Physiologically, these antibodies can be important in the case of immune evasion by certain viruses which can change their antigenic determinants by addition of small variations in the epitope regions. The immune system needs to recognize these altered antigens despite of the changes incorporated by mutations in the epitope regions. Response under such conditions may include the antibodies with broader recognition specificities. Indeed, we had observed that certain populations of mAbs do possess the additional mechanisms

Scientific Reports

and are probably designed to disregard the antigenic variations [Khan & Salunke (2014) J Immunol (2014) 192:5398]. Thus, our studies reflect degenerate recognition of the antigens, despite of subtle change, are possible in the immune system. Therefore, a systematic study of the degenerate specificity in immune recognition against constantly mutating pathogen becomes quite relevance. The dichotomy of immune evasion by fast mutating pathogen such as Influenza virus and the presence of affinity matured antibodies utilizing various mechanisms to counter antigenic variations make it an interesting puzzle. Therefore, it would be interesting to investigate this issue of multispecificity in recognition by studying the immune response against a peptide epitope of influenza virus. Studying the polyspecificity against the natural variants of the peptide epitope by screening the human phage displayed antibody libraries (Tomlinson's I and J libraries) would provide ideal model systems towards understanding the extent to which multispecificity can potentially be generated against the epitope.

Careful screening of the libraries with stringent washes had led to ten different monoclonal scFvs with specific binding to the peptide. These scFvs were established to be binding to the peptide when being expressed over phage surface and had shown binding to five out of six analogues tested with minor differences. The specific association of the scFvs expressed on phages was further confirmed by competitive ELISA using free peptide. The binding of scFvs to the peptide epitope was also confirmed by expressing the antibodies in the soluble fraction and analyzing their binding by direct ELISA. Screening of the libraries provided ten scFv clones which are diverse in sequences of CDR L2, L3, H2 and H3. Despite such diversity in the CDR residues, these clones had been able to recognize the epitope and the analogues. It was established that the degenerate specificity of these clones was similar; however they differ in their abilities to interact with the peptide as shown by competition experiments with free peptide. When compared with the individual clones isolated by hybridoma technology, these scFv clones were shown to be highly poly-specific and shown to bind not only single and double mutants but also recognized the triple mutants. Therefore, the study reveals that isolation of individual clones with very high degenerate specificity against the peptide epitope of Influenza virus is possible. Thus, isolation of such multi-reactive clones against the other conserved epitopes would be interesting in the context of the influenza neutralizing antibody problem.

We have addressed the problem of antibody degeneracy by analysis of large amount of available structural data. Over the years crystallographic studies on antigen-antibody interaction have strengthened the observation that there is no perfect apposition in the established paradigm and the emerging data. It is anticipated that a coherent analysis on global data would aid in understanding the pliable nature of an antibody. Such analyses will help understand if there is a pattern that fits the current models for speculating antigen recognition. This in turn will help understand the evolution and versatility of an antibody. The molecular mechanisms deployed during evolution of mature antibodies from a common lineage are far from clear. Diversity in recognition potential in mature antibodies is not merely a consequence of combinatorial and somatic mutational processes; rather antigens also tend to modulate evolution of an antibody. To advance our knowledge of immune recognition by humoral immune system explicit understanding of the processes of B-cell development becomes imperative. In the light of this, we have compiled antibody-antigen crystallographic data from mouse from RCSB PDB.

Extensive structure and sequence analyses have shown deviation in the established paradigms of specificity associated with antigen recognition resulting in several interesting observations. Role of CDRH3 in recognition as well as interaction with antigen

has been reported in various studies [Jiang et al., Nat. Struct. Mol. Biol. (2010); 17: 955, Calarese et al., Faseb J. (2008); 22: 1380]. A systematic analysis of the structures as well as sequences of compiled data from mouse has shown that length as well as sequence of H3 varies significantly amongst mature antibodies of a common lineage. Variation in length suggests possible INDEL within the CDR during maturation event. Those antibodies with long H3 loop primarily bind to protein antigens while a few bind to hapten and sugar. Variation in sequence is also reflected in topology of the H3 loop. Structure superposition as well as structure based sequence alignment of the mature antibodies has shown that in 58% of data, RMSD of CDRH3 is above 2 Å, followed by 39% of the data with RMSD in the range of 1-2Å, while for 3% of the data RMSD of less than 1 Å is seen. Rest of the heavy chain CDRs do not exhibit any distinct feature and they superpose quite well suggesting similar geometry of the loops. Interestingly, CDRL1 has also been seen to be structurally diverse in the compiled data as in 98% of the data RMSD of CDRL1 is >1 Å. Variation in length is also a common attribute of L1. Rest of the CDRs of light chain do not exhibit any distinct feature except in some cases where L3 varies in length and loop topology.

No complete alignment in the antigen could be obtained for any of the sets as each of the mature antibodies was bound to distinct epitope. For those sets where all the antigens were proteinaceous, no immune-dominant epitope was present. In as many as 6 groups, antibodies bound to antigens with significant chemical and structural diversity. These epitopes were seen to accommodate overlapping sites within the paratope. To gain further insights into the dynamics involved in multiple recognition behaviour of antibodies of a common lineage, optimization of all-atom simulation of the complexes is underway.

Diverse recognition potential of antibodies originating from common germline lineage is a consequence of major structural change in the backbone of CDRH3, CDRL1 and to some extent of CDRL3. Insertions and deletions in H3 is a key dictating factor. However, in a few cases where antibodies bind to common epitope, minimal side-chain rotameric movements have been seen.

As a part of our structural proteomics of seed allegen studies, we had reported earlier the crystal structure of SM80.1 and SM80.2 from eggplant (*Solanum melongena*). These structures have now been refined and structure–function analyses carried out. While SM80.1 shows possible allergy specific determinants within the protein, certain bound lipidic entities were detected in SM80.2 which may be responsible for its allergenic properties. Expanding these studies further, proteins of other seeds are also being explored. The seeds of *Buchanania lanzan* (Chironji) are treenuts of the family Anacardiaceae. The protein content of the seeds was subjected to salt fractionation over a wide concentration range of ammonium sulphate and further separated by SDS-PAGE and N-terminal sequencing of the dominant proteins was carried out. Two proteins, namely, BL1 and BL2 were identified to be homologous to P54 protein (*Pisum sativum*) and Vicillin (*Pistacia vera*), respectively. The BL3 has been purified and the crystallization attempts are in progress.

Future plans

Bioinformatics and crystallographic analyses of antigen-antibody recognition as well as broader aspects of host-pathogen interactions will be continued with the ultimate goal to correlate the structural principles with physiological implications. Structural proteomics of plant allergens will be continued towards crystallographic analysis and structure-function correlation.

Scientific Reports

Publications

1. Jain A and Salunke DM (2015) Purification, identification and preliminary crystallographic studies of an allergenic protein from *Solanum melongena*. ***Acta Crystallogr*** F71:221.
2. Kaur H and Salunke DM (2015) Antibody promiscuity: Understanding the paradigm shift in antigen recognition. ***IUBMB Life*** 67:498.

Modulation of host immunity and nutrient allocation by a biotrophic pathogen

Divya Chandran

Young Investigator
Rashi Gupta

Ph. D. Students
Megha Gupta
Arunima Gupta

Research Theme

Obligate biotrophic pathogens like the powdery mildew fungi manipulate living plant hosts to acquire nutrients while limiting host defence responses. Powdery mildew is one of the most devastating diseases limiting productivity of food legumes in India and other developing countries. Chemical methods used to control the disease are neither economical nor environmentally sustainable; therefore, there is a need to develop innovative strategies to combat this disease. To date, few powdery mildew resistant legume varieties have been identified, with the identity of the genes conferring resistance and the underlying molecular mechanisms not known. We aim to elucidate the molecular mechanism(s) underlying the legume-powdery mildew interactions via infection site-specific analyses in the *Medicago truncatula-Erysiphe pisi* pathosystem. Our long-term goal is to engineer durable powdery mildew resistance in food legumes of agronomic import.

Objectives

The major goal of our research is to identify and target novel legume host genes associated with defence that limit growth of the pathogen as well as host compatibility factors required for the growth and reproduction of the pathogen. Conversely, we are also interested in identifying pathogen effector proteins, which may target host basal defence responses or provide access to host nutrients. We envisage 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 pathosystem:

- Identification of novel infection site-specific host components, cellular processes, and pathogen effectors impacting powdery mildew proliferation
- Understanding the role of host basal defence responses in limiting powdery mildew growth
- Elucidation of key factors mediating carbon (re)allocation at the host-pathogen interface

Scientific Reports

Progress

Powdery mildew fungi are obligate biotrophic pathogens that can only propagate on living plant cells. Powdery mildew disease can reduce pea and mung bean yields by 25-50% and is emerging as a significant pathogen on chickpea. These fungi grow superficially on leaves, stems, and sometimes fruits and are visible as a fine white powder on the host surface (Fig. 1). Figure 1A diagrams the progression of powdery mildew infection by *Erysiphe pisi* on leaves of the model legume *Medicago truncatula*.

Germination of the conidium occurs within a few hours post inoculation (hpi), followed by penetration of the host epidermal cell requiring the formation of multi-lobed appresoria. The penetration peg invaginates the epidermal cell plasma membrane and then differentiates into the haustorium. Development of the haustorial complex, comprised of the haustorium, extrahaustorial matrix and extrahaustorial membrane, occurs within 24 hpi. The intricate haustorial complex is required for nutrient uptake by the fungus. Further growth and reproduction of the fungus results in the development of surficial hyphae, conidiophores, and conidia (by 5 dpi; Fig. 1) on the aerial epidermal tissue. At 7-10 dpi, the powdery mildew is clearly visible to the naked eye on infected leaves. Wind-dispersed asexual conidia rapidly propagate new infections.

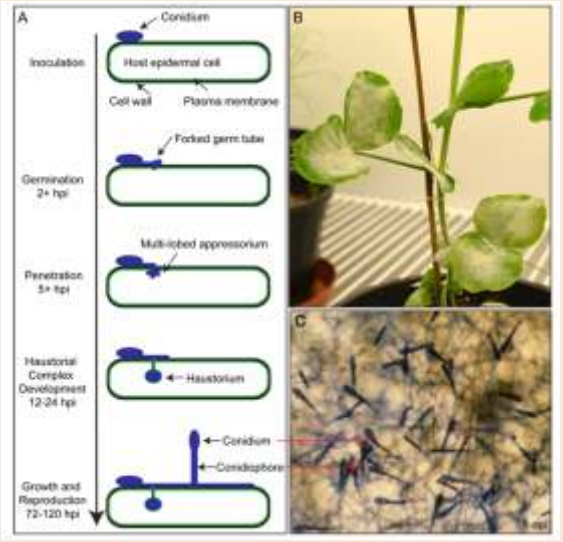


Figure 1. Powdery mildew disease of legumes. (A) Schematic of powdery mildew infection process. (B) Powdery mildew visible as a white powder on surface of pea leaves. (C) Fungal asexual reproductive structures visualized using trypan blue dye at 5 dpi on a susceptible M. truncatula accession.

To pursue our research objectives, our first priority was to obtain a pure isolate of the *E. pisi* pathogen capable of infecting both pea and *M. truncatula*. Since *E. pisi* is an obligate biotroph, it cannot be cultured and preserved on artificial media, and is therefore not available at fungal culture collections centers (e.g. Indian Type Culture Collection, IARI). Hence, we performed online searches for Indian laboratories or agricultural stations working with pea powdery mildew, where the pathogen would have to be routinely maintained on a living host. After contacting several labs, we were finally able to obtain a pure isolate of *E. pisi* from the laboratory of Dr. D. K. Banyal located at the CSK Himachal Pradesh Agricultural University, Palampur. The isolate was named Tanda, as it was originally obtained from powdery mildew growing on pea plants in the Tanda region of Himachal Pradesh.

1. Identification of novel infection site-specific host components, cellular processes, and pathogen effectors controlling powdery mildew proliferation

The structural development and progression of powdery mildew infection on the host plant is well defined, highly localized, and restricted to the epidermal cell. Likewise, host responses to the pathogen have also been shown to be localized to a few cells surrounding the haustorium-infected cell. To increase our sensitivity at detecting such localized responses and enable the identification of novel host defence and pathogen compatibility genes, we will employ laser capture microdissection (LCM) in combination with next generation sequencing (NGS) to specifically profile the transcriptome of the infection site.

We first aimed to identify optimal time points after *E. pisi* inoculation for use in resolving gene expression patterns linked to pathogen growth. In addition, we aimed to identify *M. truncatula* accessions exhibiting varying degrees of resistance/susceptibility to the pathogen, which would allow us to dissect host defence responses from compatibility factors (vital for the survival and proliferation of the pathogen). We tested the degree of pathogenicity or virulence of the *E. pisi* Tanda isolate by infecting six different M. truncatula accessions previously known to exhibit varying degrees of resistance or susceptibility to other isolates of the pathogen. We performed a time course macroscopic (visual inspection of symptoms) and microscopic evaluation of *E. pisi* growth on the different accessions. Based on our observations were able to classify the accessions as resistant (R), moderately resistant (MR), moderately susceptible (MS), or susceptible (S) to the Tanda isolate of *E. pisi*. Profuse conidiation was visible on S accessions between 5-7 dpi. In contrast, growth of the pathogen was arrested either at the hyphal stage, appresorial stage, or forked germ tube stage in the MS, MR and R accessions, respectively.

Next we aimed to develop a method for preparing *M. truncatula* mature leaf tissue for use with LCM. LCM is a technique which allows for individual cells or groups of cells to be isolated from heterogenous tissue sections while they are viewed under a microscope. Harvested cells then provide RNA for the profiling of gene expression from individual cell types. For LCM, it is critical that plant tissue be prepared in a way that leaf internal structure is preserved and that RNA isolated from these cells is of sufficiently high quality and yield for downstream gene expression profiling. Three chemical fixatives were compared using the conventional paraffin method: Farmer's fixative (ethanol:acetic acid, 3:1), Methacarn (absolute methanol:chloroform:glacial acetic acid 6:3:1) and 100% Methanol. Briefly, infected and uninfected leaves were dissected into small pieces and transferred immediately into separate vials containing different fixatives and incubated at 4°C overnight. Tissues were subsequently dehydrated in a graded series of ethanol and embedded in liquid paraffin wax (Paraplast-X-Tra). Individual leaf pieces were then positioned within paraffin blocks, sectioned using a microtome, stretched and air-dried. Sections were then deparaffinized and viewed under a bright field microscope. We found that farmer's fixative (FF) provided the highest preservation of leaf internal structure, as evidenced by the presence of fully expanded and rounded epidermal cells, and mesophyll cells with intact chloroplasts. Furthermore, we were also able to clearly visualize fungal haustoria within epidermal cells of FF fixed infected *M. truncatula* leaf tissue. In contrast, the use of methacarn as a fixative resulted in compressed and broken epidermal cells, while the use of 100% methanol provided inconsistent results.

2. Elucidation of key factors mediating carbon (re)allocation at the host-pathogen interface

Biotrophic pathogens like the powdery mildew fungi acquire nutrients, especially sugars, from their host through fungal feeding structures termed 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. Since this alteration in the source-sink relationship at the powdery mildew infection site is critical to the growth of the pathogen and few molecular components are known, our aim is to identify key players and regulators mediating this response.

Scientific Reports

Scientific Reports

It is well known that sucrose is the primary sugar transported from source to sink organs. While apoplastic sucrose may be utilized by the pathogen, there is evidence to suggest that glucose is the major carbon that is imported from the host and is essential for growth and metabolism of the fungus. Accumulation of hexoses at the plant-biotroph interface may be facilitated by plant and fungal cell wall invertases. Notably, these hexoses may not only serve as a carbon source for pathogen growth, but may also be important in maintaining sink strength by effectively lowering sucrose concentrations in these tissues.

Recently, a new class of sugar efflux transporters, known as SWEETs (Sugar will eventually be exported transporters), has been implicated in pathogen nutrition (reviewed in Chandran 2015, IUBMB Life). In plants, SWEETs play roles in various developmental processes where sugar efflux is essential, including sucrose loading of phloem for long-distance sugar transport, nectar secretion, embryo and pollen nutrition, and maintenance of sugar homeostasis in plant organs. Eukaryotic SWEET proteins encode membrane-localized uniporters that transport sugars across cell membranes down a concentration gradient. Notably, these transporters have been shown to be selectively targeted by pathogens to gain access to host sugars. 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. These bacteria utilize their type III secretion system to secrete transcription-activator like (TAL) effectors to directly target the expression of specific *SWEET* genes. In most cases, 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 powdery mildew) modulate the expression of plant *SWEET* genes. In most cases, it has not yet been established whether the infection-triggered expression of *SWEET* genes promotes pathogen growth and virulence. However, there is growing evidence to suggest that co-option of SWEETs may be a universal strategy adopted by diverse types of pathogens to divert sugars from their host. Therefore, one of our objectives is to elucidate whether M. *truncatula* SWEETs play a role in powdery mildew nutrition.

First, using a bio-informatics approach we identified ~24 SWEET protein candidates in M. *truncatula*. Only proteins with two MtN3/Saliva motifs, characteristic of eukaryotic SWEETs, were considered as candidates. We then constructed a phylogenetic tree of plant SWEET proteins using *Arabidopsis*, rice, grape and M. *truncatula* protein sequences and found that they fall into four subclades. Previous work by other labs has shown that Clades I, II, and IV members function primarily as monosaccharide efflux transporters, transporting glucose, galactose, and/or fructose, whereas Clade III members preferentially transport the disaccharide sucrose.

Since Clade III SWEET transporters were previously implicated in *Xanthomonas* nutrition and have also been shown to be induced in response to the powdery mildew fungus in *Arabidopsis*, we hypothesized that Clade III SWEETs may play an important role in promoting powdery mildew growth on legumes. We quantified expression of Clade III *MtSWEETs* via quantitative PCR at different time points after *E. pisi* inoculation. Preliminary data identified a number of Clade III *SWEETs* with elevated expression (3-25 fold) in the S accession in response to *E. pisi* inoculation. Moreover, we found that expression levels peaked just prior to onset of fungal conidiation. Interestingly, a number of Clade I *SWEETs* were also induced in the S accession (10-90 fold) in response to infection. To determine

whether the elevated *MtSWEET* gene expression in the S accession is related to pathogen growth, we quantified expression of these genes in the R accession. We hypothesized that if *MtSWEET* expression is required for pathogen growth then pathogen-induced expression would either be absent or limited in the R interaction. We found that expression of Clade III and I *MtSWEETs* was lower in the R accession. Furthermore, these *MtSWEETs* exhibited peak expression at earlier time points post *E. pisi* inoculation. It is well known that sugars act as signaling molecules that can regulate defence mechanisms via the control of gene expression. For example, sugars induce pathogenesis-related genes and repress photosynthesis. Therefore, from the host's perspective, early induction of *SWEETs* may result in the activation of defence responses that would help limit the growth of the pathogen. Indeed, in support of our hypothesis, we observed enhanced expression of a host pathogenesis-related gene, *PR10*, at early time points of PM infection only in the R accession.

Future plans

One of the major issues of using the conventional paraffin tissue preparation method for LCM is the time required for proper paraffin infiltration (7-9 days), increasing the chances of nucleic acid degradation. This is particularly problematic for our LCM application, since the starting sample size will be extremely small (e.g. haustorium-infected epidermal cells). For better preservation of integrity of RNA isolated from these laser microdissected cells, we plan to optimize a microwave paraffin method that will facilitate even and rapid cell permeation and reduce preparation time to ~ 5 hours. Next we will harvest individual cell types at the infection site using LCM and profile the transcriptome at various time points after pathogen inoculation in *M. truncatula* accessions with altered powdery mildew resistance. A challenge of performing infection site-specific profiling in R interactions is visualization of infection sites in the absence of well developed fungal structures. While fungal haustoria can be readily detected in S accessions using simple staining techniques, infection sites would be difficult to visualize in R interactions, where the growth of the pathogen is arrested at stages prior to haustorium formation. To be able to visualize the infection site in R accessions, we aim to develop a simple staining technique using dyes that would detect specific constituents of cell wall appositions (or papillae) that are formed as a host defence response to attempted fungal penetration. Furthermore, to facilitate downstream processing of NGS data we will formulate a bio-informatics plan to distinguish fungal sequences from plant sequences and also collate transcriptome data from EST libraries of both host and pathogen.

To ascertain the role of MtSWEET candidates in powdery mildew nutrition, we will perform functional assays using mutant, infection site-specific knockdown or over-expression lines in *M. truncatula* and/or *Arabidopsis*, as needed. We anticipate that altering the expression of genes essential for nutrient acquisition specifically at the site of infection will limit sugar availability and consequently inhibit pathogen growth. In addition, we will also identify and characterize cell wall invertases and hexose transporters that may be involved in carbon partitioning at the host-pathogen interface.

Publications

1. Chandran D, Scanlon MJ, Ohtsu K, Timmermans MCP, Schnable PS and Wildermuth MC (2015) Laser microdissection-mediated isolation and in vitro transcriptional amplification of plant RNA. ***Curr Protoc Mol Biol*** 112:25A.3.1.
2. Chandran D (2015) Co-option of developmentally regulated plant SWEET transporters for pathogen nutrition and abiotic stress tolerance. ***IUBMB Life*** 67:461.

Scientific Reports

Pathophysiology of procoagulation, inflammation and thrombosis in different diseases and immune responses

Prasenjit Guchhait

Collaborators
Tulika Seth (AIIMS)
Naval K. Vikram (AIIMS)
Kavita Chatterjee (AIIMS)
Parvaiz Kaul (SKIMS)
Subodh Kumar, (AIIMS Trauma Centre)
J. C. Suri (Safdarjang Hospital)
M. K. Sen (Safdarjang Hospital)
Satyajit Rath (NII)
Vineeta Bal (NII)
Shinjini Bhatnagar (THSTI)
P. Thiagarajan (Baylor College of Medicine)
Josef T. Prchal (University of Utah)

Young Investigator
Sheetal Chawla

Postdoctoral Fellow
Avinash Gupta

Ph. D. Students
Amrita Ojha
Angika Bhyasam
Sulagna Bhattacharya
Gowtham K Annarapu
Rashi Singhal
Swarnima Pandey

Project Staff
Dipika Nandi

Research Theme

The delineation of complex pathophysiology of procoagulation, inflammation and thrombosis, and immune responses in different disease conditions. Current research projects aim to elucidate the following mechanisms: 1) how intravascular hemolysis increases the risk of thrombosis and thromboembolism in hemolytic disease such as paroxysmal nocturnal hemoglobinuria (PNH); 2) Development of systemic inflammation in trauma: role of hemolysis; 3) Hypobaric hypoxia regulation of procoagulation and thrombosis, and immune functions of native highlanders; 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.

Scientific Reports

Progress

1. We have demonstrated that the binding of extracellular hemoglobin (Hb) to glycoprotein (GP)1bA on platelet surface induces its activation and apoptosis in a concentration-dependent manner. *In vitro*, the lower Hb concentrations (0.37-3 mM) activate platelets and higher concentrations induce apoptosis, and in turn promote platelet aggregation and clot formation (Figure 1). The plasma extracellular Hb or platelet surface bound Hb correlates with platelet activation in PNH patients. This work is now at the stage of 2nd review in a peer-reviewed journal.

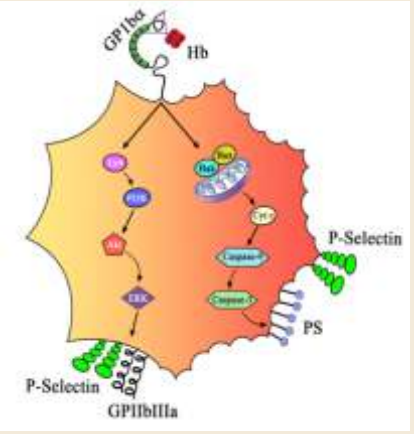


Figure 1 Hb at lower concentrations (0.37-3 μM) induces inside out signaling via Lyn, PI3K, AKT, and ERK by binding to GP1bα and increases surface expression of P-selectin and GPIIb-IIIa. On the other hand, concentrations of Hb (3-6 μM) induce apoptosis and increase expression of Bak and Bax, release cytochrome C, activate caspase-9 and caspase-3, and expose PS on platelets.

2. We are investigating the role hemolysis in the progression trauma complications. The pathophysiology of systemic inflammatory syndromes in trauma victims is quite complex. Since hemolysis is a crucial part of injury we are investigating how the neutrophils and monocytes contribute to the above pathophysiology after uptaking cell-free Hb. We observed significant changes in function and differentiation of the above cells in hemolytic conditions. This work is under progress currently.

3. Recently we have shown that novel mutations in a hypoxia responsive gene, EGLN1 that supports normal erythropoiesis in Tibetans who are living at high-altitude hypoxic conditions for generations [Lorenzo et al (2014) Nature Genetics 46:951]. This phenomenon is just opposite when lowlanders travel to high-altitude they develop polycythemia due to hypoxia exposures.As the extension of the above study we are currently investigating if these highlanders have normal coagulation and homeostasis mechanisms unlike the lowlanders when travels to high altitude, they develop procoagulant states, edema and thrombosis. We observed that *in vitro* knockdown of hypoxia inducible factor (HIF-1A) and early growth response factor (EGR-1) in monocytes downregulates procoagulat tissue factor (TF) and anticoagulant plasminogen activator inhibitor (PAI-1). Whereas under hypoxia treatment, HIF-1A or EGR-1 upregulated TF and PAI-1 in monocytes. The hypoxic exposure to monocytes promoted plasma clotting. Using the proteomic approach (by 2-dimensional gel electrophoresis and MS analysis) we show that hypoxia treatment to monocytes up/down regulates several pro/anti coagulation factors and their precursor molecules. We are now working on the mechanisms whether the HIF-1A and EGR-1 are associated with the regulation of these pro/anti coagulation factors. Parallely, we are pursuing the assessment of pro/anti coagulation/thrombotic factors in highlanders, with and without mutations at EGLN1 and EPAS1 genes.

So with given evidences of mutations at EGLN1 and EPAS1 (Lorenzo et al 2014), we sought to investigate manifestations of these mutations in the immune cytome of the Tibetan highlanders. Preliminary *in vitro* studies have shown that monocyte markers like HLA-DR, CD18, CD14, CD16 etc vary upon exposure to 5% oxygen. Also, upon differentiation of THP1 into macrophages under hypoxia they display a different phenotype from that of normoxia. We are now trying to understand the effect of these adaptive mutations on the differentiation of THP1 cells to immune cell lineages under different hypoxic conditions. We are in the process of assessing the hypoxia and immune cytome in native Tibetan at high-altitude compare to their counterparts at sea level.

4. We are elucidating the mechanism(s) underlying the pathogenesis of thrombocytopenia in dengue infections. We have collected blood samples from NS1

Scientific Reports

antibody positive dengue patients during different days of infections. Our data (from 7 dengue patients at day-1, day-3 and day-5 of hospitalization) shows a trend of platelet activation during different days of infections. The platelet activation markers such as P-selectin, PS, GPIIbIIIa and GP1bA are inversely correlated with platelet counts in circulation. We are collecting more samples currently, and measuring more parameters such as IgG/IgM and complement factor C3 on platelet surface along with the above parameters. We are examining whether activation of platelets help the antibody/complement factor binding to platelet surface, which finally clear the platelets from circulation. We are measuring different transcription factors (such as Runx1, Gata1 and Fli1) associated with differentiation of hematopoietic stem cell (HSC) to megakaryocyte (MK) in patients using intracellular staining and FACS analysis of their PBMCs. This will explain if the less platelet production by MK cells is associated with thrombocytopenia in these dengue patients.

5. The complement factor H (CFH) and the CFH-related (CFHR) proteins play very important roles in defence mechanism by enabling accumulation of activated complement components on pathogenic surface while preventing complement activation on host cell surfaces. CFHR1 deficiency in some cases leads to an autoimmune condition in disease such as atypical hemolytic uremic syndrome (aHUS) characterized by severe kidney pathology and often associated with presence of autoantibody against CFH, whereas in many other cases CFHR1 deficiency remains asymptomatic. Studies reveal that such cases of asymptomatic CFHR1 deficiency occurs in human population around the world including India at a frequency of 10%. We have designed experiments to understand if the immune cell subsets in these asymptomatic CFHR1 deficient individuals can provide clues for understanding malfunctioning of the innate and adaptive immune components in the absence of CFHR proteins, which might skew the system towards autoimmunity. Our data show that CFH proteins bound preferentially to the surface of classical and inflammatory monocytes, but not to other subtypes of monocytes. Further data shows that CFH protein bound significantly to classical monocytes of the CFHR1 deficient individuals (n=4). With the above observations we are now investigating the particular role of CFH in monocyte functions and differentiation in the CFHR1 deficient individuals.

6. We are pursuing a collaborative project to understand the distinct developmental and functional properties of the neonatal immune system and their clinical consequences in the neonatal period. We are investigating the differences in the cord blood immune phenotypes (particularly monocyte subsets, 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. Our initial data show that SGA neonates have comparatively fewer plasmacytoid dendritic cells (pDCs), a higher myeloid DC (mDC) 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. Our plan is to follow the above observations in detail. We 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.

Future plans

- Understanding the effects of intravascular hemolysis on the differentiation and development of different hematopoietic cell lineages in the disease pathogenesis.
- Assessing whether the intrauterine hypoxic conditions in mothers (mostly suffering from hypertension, asthma, tuberculosis or such diseases) affects the hypoxia responsive signals and the development of immune systems in preterm neonates.

Publications

1. Singhal R, Annarapu GK, Pandey A, Chawla S, Ojha A, Gupta A, Cruz MA, Seth T and Guchhait P (2015) Hemoglobin interaction with GP1ba induces platelet activation and apoptosis: a novel mechanism associated with intravascular hemolysis. ***Haematologica*** (in press).
2. Da Q, Teruya M, Guchhait P, Teruya J, Olson JS, Cruz MA (2015) Free hemoglobin increases von Willebrand factor-mediated platelet adhesion in vitro: implications on circulatory devices. ***Blood*** (in press).
3. Pandey A, Chawla S and Guchhait P (2015) Type-2 Diabetes: Current Understanding and Future Perspectives. ***IUBMB Life*** 67: 506.

Effector-triggered Immunity: Elucidating inositol-regulated pathways in assemblies and signaling by immune regulators

Saikat Bhattacharjee

Young Investigator
Bornali Gohain

Postdoctoral Fellow
Jewel Jameeta Noor

Ph. D. Students
Hitika Gulabani
Ingole Kishor Dnyaneshwar
Krishnendu Goswami
Mritunjay Kasera

Research Theme

Effector-triggered immunity is induced upon sensing specific effector activities (termed avirulence factors; Avr) on either cognate resistance proteins (R) directly or on specific host target (guardee) which the R protein guards. These effector activities mostly fall under the broad category of post-translational modifications (PTMs) of host targets. In current understandings, the robust and rapid effector-triggered immunity (ETI) involves crosslinked and intricate signaling networks that leads to massive transcriptional reprogramming. The routes by which the transcriptome changes are achieved remain unknown. Our research focus on understanding this regulation and trigger of immune signaling using the *Arabidopdis thaliana-Pseudomonas syringae* pv tomato (DC3000) pathosystem.

Objectives

Conventional modes of linear arrangement of transducers and receivers that characterize most signaling cascades are likely not followed during ETI. Instead ETI is characterized by crosstalks among multiple networks that aid to communicate the signal rapidly towards massive transcriptional reprogramming. During trigger, effector activities post-translationally modify host targets and as a result alter regulated associations of key immune player including the R proteins. This in turn activates the R protein from its inactive state. Thus, ETI is often considered an accidental consequence of effector functions. Some of our earlier work has identified membrane localized complexes containing R proteins (such as RESISTANCE TO *PSEUDOMONAS SYRINGAE* 4 or 6; RPS4 or RPS6) with regulators of immunity such as SUPPRESSOR OF RPS4-RLD 1 (SRFR1) and ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1). Specific interactions in this complex are disrupted in the presence of effectors such as AvrRps4 and HopA1 likely suggesting the gateway towards ETI signaling. However, neither the detailed molecular mechanism of immune assemblies nor the mode of these effector functions through which the immune complexes are altered and downstream signaling coupled have been deciphered. Our broad goal is to unravel these processes.

We are focused on the role of inositol derivatives in the assembly of defence proteins and signaling during ETI. Inositols are versatile molecules in their nature of polarity (Figure 1). As polar inositol phosphates (InsPs), they define secondary messengers in eukaryotic signal transductions responsible for plethora of cellular processes such as hormone signaling, transcriptional regulation and apoptosis. However their role in immunity remains to be detailed. Pathogen invasion has been reported to trigger phospholipases (PLCs) which generate inositol phosphates from lipid-conjugated inositols, the non-polar phosphatidylinositols (PtdIns), present on membranes. These lipid-modified inositols define membrane architectures and impart signatures to organelles. An instance where a pathogen utilizes PtdIns to access a cell has also been reported. Our investigations into roles of InsPs and PtdIns involve the following approaches:

- Elucidation of metabolic profiles of inositol-phosphates (InsPs) in plant mutants altered in defence responses in order to identify specific signaling routes.
- Identifying steady-state protein-protein interactions platforms of resistance proteins and immune regulators on lipid interfaces in the plant cell and how pathogen effectors modulate signaling.
- Identifying inositol compound-dependent synergistic and antagonistic cross-talk between hormonal pathways and how pathogen effectors or induced ETI impinge of this network.

Scientific Reports

Progress

In published reports close parallels have been drawn between inositol biosynthesis/metabolism mutants to responses during innate immunity. Of special importance is the *Arabidopsis* MIPS1 (a type member of the myo-INOSITOL PHOSPHATE SYNTHASE family). Plants harboring homozygous mutation in MIPS1 (*mips1-2*) display

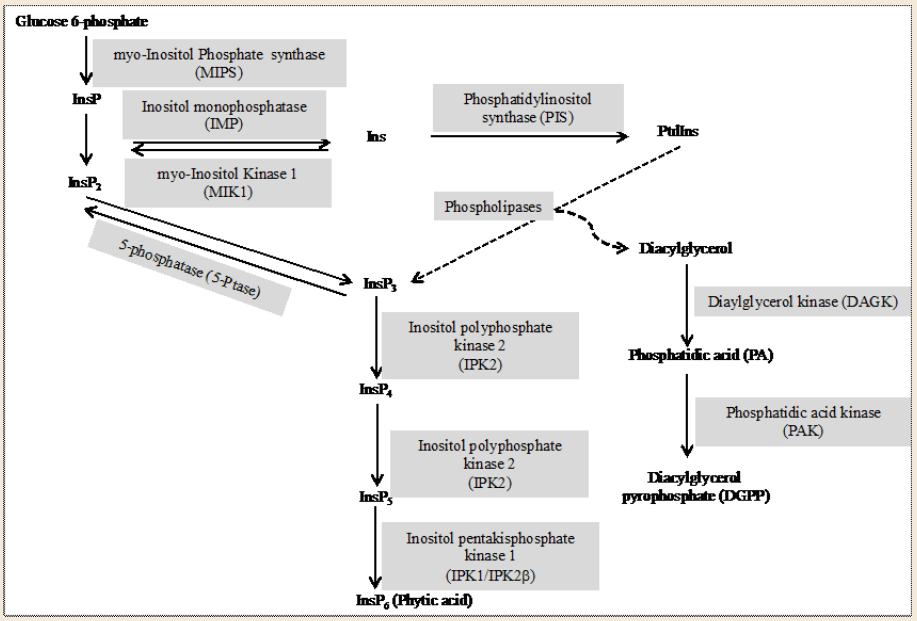


Figure 1: Inositol biosynthesis and metabolic pathway. Cellular synthesis of inositol phosphates (InsPs) and phosphatidylinositols (PtdIns) are shown. Enzymes catalyzing key steps are shaded grey.

spontaneous cell death, reminiscent of hypersensitive response that sometimes associate with ETI. Positive defence modulators such as EDS1 and defence hormones such as SA are upregulated in *mips1-2* plants suggesting that MIPS1 is a negative regulator of defence. Other reports have suggested that by altering relative levels of different inositol phosphates, defence responses are modulated. We are utlizing a constitutively active *srfr1-4* plants as a system to understand roles of inositols in ETI. In qPCR assays, several InsP biosynthesis and metabolism genes are significantly altered in *srfr1-4* (Figure 2a).

The molecular details of enhanced resistance displayed by the *srfr1-4* plants remain to be explored. Possible consequences in *srfr1-4* include altered relative levels of different InsPs and/or unstable assemblies of immune regulators allowing for unregulated defenses. Interestingly, gene expression database searches identifies *SRFR1* is co-regulated with *INOSITOL PENTAKISPHOSPHATE KINASE 1 (IPK1)*, a enzyme involved in the biosynthesis of phytic acid (InsP6). We have obtained T-DNA tagged mutant plants of IPK1 (*ipk1-1*). These plants are slightly stunted in appearance when grown under long day conditions and resemble growth phenotype of the *srfr1-4* plants (Figure 2b). We show that IPK1 transcripts are reduced in *srfr1-4* and genetically *srfr1-4* is epistatic to *ipk1-1* (Figure 2). In preliminary assays *ipk1-1* is enhanced resistant to DC3000 suggesting that genetically IPK1 or InsP6 functions as a negative regulator of defense. Whether lower levels of InsP6 or accumulation of lower InsPs (such as InsP5, InsP4 and InsP3) in *ipk1-1* and *srfr1-4* accounts for the enhanced resistance is being investigated. In a recent progress we have

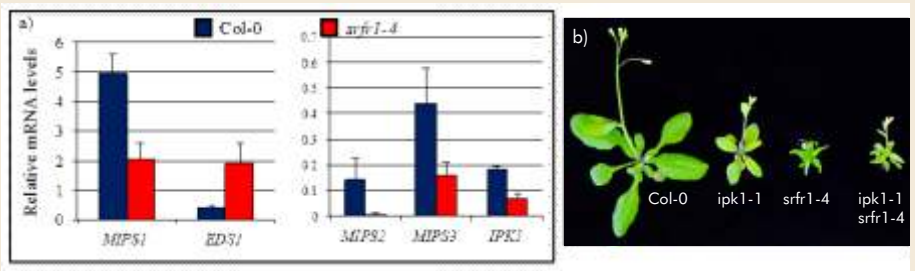


Figure 2: Inositol biosynthesis and metabolism genes have altered expression in srfr1-4 plants. (a) qPCR of MIPS and IPK1 Col-0 (wild-type) versus srfr1-4. (b) Growth defects in ipk1-1 and srfr1-4. A double mutant ipk1-1 srfr1-4 resembles srfr1-4 plants suggesting srfr1-4 is epistatic to ipk1-1.

been able to successfully resolve myo-inositol, InsP2 and InsP3 by anion exchange chromatography. Separation and identification of higher InsPs are in progress. A direct effect of alteration in different InsPs ratios cause changes in defense-related phytohormone responses such as those mediated by salicylic acid (SA) and jasmonic acid (SA). We are generating plants in which one or more phytohormone signaling are disrupted. Whether disease responses of *ipk1-1* are rescued is being tested.

A recent report identified mRNA export defects in *ipk1-1* plants. Protein-protein interactions mediated by IPK1 via a cofactor role of its byproduct InsP6 regulate mRNA export. Nucleoporins, localized at the nuclear pores, function centrally in mRNA and protein export from the nucleus. Altered defenses in certain plants have been associated with defects in mRNA export caused by mutations in nucleoporins. An obvious consequence of regulated mRNA export would affect the translation efficiencies of both positive and negative defence modulators. Whether such regulations occur during ETI remains to be identified. Towards this we are testing the *srfr1-4* plants for such defects. In addition we are in the process of generating chemical-inducible (Dexamethasone) transgenic expression lines of avirulent effectors AvrRps4 and HopA1. These plants will provide us a system to induce and track ETI in a controlled time-dependent manner. One of our goal is to study the steady state and upon dexamethasone treatment, the nucleocytoplasmic partitioning of defence-related mRNA.

Immune assemblies of RPS4, EDS1 and SRFR1 are anchored on membranes via unknown mechanisms since none of these proteins contain predicted transmembrane domains. Likely these associations on membranes are facilitated by binding to phosphatidylinositols (PtdIns). How avirulent effectors AvrRps4 and HopA1 perturb these assemblies are not known. In a recent advancement the bacterial effector HopA1 was reported to bind PtdIns. We are expressing EDS1 and SRFR1 in heterologous systems to test for PtdIns binding in *in vitro* and *in vivo* assays. Localization of protein assemblies especially on membranous compartments may also require additional post-translational modifications. The identity of these modifications is being tested *in planta*. We have generated epitope-tagged SRFR1 and EDS1 which functionally complement defects in their respective mutants. These plants are being genetically combined with dexamethasone-inducible ETI lines to identify pre- and post-ETI changes in their PTMs. As a sub-part of these studies we are pursuing a collaboration with Dr. Walter Gassmann, University of Missouri-columbia, USA. These investigations involve protein SUMOylation and their role in protein-protein interactions. In recent observations, eds1 containing a lysine to arginine substitution (K478R) in the predicted SUMOylation motif is non-functional in immunity. We are currently optimizing *in vitro* and *in vivo* SUMOylation

Scientific Reports

detection assays to test for SUMOylation of EDS1. Several other defence modulators either possess SUMOylation motif or can bind SUMO/SUMOylated proteins. The identity and role of these defence modulators are being investigated.

Future Plans

- We will continue the further molecular characterization of *ipk1-1* and other inositol biosynthesis and metabolism mutants. These investigations include testing for altered defence responses, qPCRs for defence-related genes and generating combinatorial mutants with plants deficient in immune responses. This approach will identify genetic links between InsPs and defence players. Our priority is to understand molecular defects (such as mRNA export) in *ipk1-1* that causes enhanced resistance and further dissect its possible genetic link to SRFR1.
- We will optimize ion-exchange chromatography based separation and quantification of InsPs in plant extracts. Once optimized InsPs will be first compared among wild-type, *ipk1-1* and *srfr1-4* to gain better understanding of relative changes in different InsPs upon defence responses.
- Unlike animal systems, the receptor of InsP3 that facilitates Ca^{2+} release from intracellular stores has not been identified. Current understanding suggests that InsP3 in plants is converted to InsP6. We have initiated a collaboration with Dr. S.V. Eswaran, Emeritus professor, RCB to synthesize fluorescence or matrix-conjugated InsP6 in order to identify InsP6-binding proteins in plants.

Publications

1. Bhattacharjee S, Noor JJ, Gohain B, Gulabani H, Dnyaneshwar IK, and Singla A (2015) Post-translational modifications in regulation of pathogen surveillance and signaling in plants: The inside - (and perturbations from) outside story. ***IUBMB Life*** 67: 524.

Intrinsic Signals that Regulate Skeletal Muscle Structure and Function

Sam J. Mathew

Young Investigator
Masum Saini

Ph. D. Students
Megha Agarwal
Pankaj Kumar
Akashi Parashar
Shreyasi Das

Project Staff
Amit Kumar
Ankur Kumar

Research Theme

We are interested in understanding the process of cellular differentiation and its regulation. We use the skeletal muscle as the tissue of interest, focusing 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.

Objectives

The major goal of our research is to understand the molecular basis of cellular differentiation, and how aberrant differentiation leads to diseases, using the skeletal muscle, for which we have the following objectives:

- Document the temporal expression dynamics of a family of skeletal muscle specific genes called skeletal muscle myosin heavy chains (MyHCs), critical to muscle structure and function, during mouse embryonic, fetal and perinatal development.
- Study the function of specific MyHCs in vivo during mouse development by generating specific MyHC conditional targeted mouse alleles, and in vitro using gene knockdown in myogenic C2C12 cells.
- Identify genes with dynamic expression characteristics during C2C12 myogenic differentiation, and investigate their functional requirement in myogenesis.
- 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.

Progress

Myosins are motor proteins important in numerous cellular processes such as cell motility, cell division and transport of cargo within cells. There are multiple classes of myosins and one of them is the Class II myosins which comprise myosins critical for skeletal muscle contraction. Each such skeletal muscle contractile myosin molecule is a heterohexamer, comprising a pair of Myosin Heavy Chains (MyHCs), a pair of Myosin Essential Light Chains and a pair of Myosin Regulatory Light Chains. There are multiple MyHC isoforms in mammals, with most expressed during adult stages, where they facilitate proper muscle contraction. Interestingly, three MyHC isoforms, namely MyHC-embryonic, -perinatal and -slow 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

Scientific Reports

adult life. MyHC-embryonic and -perinatal are re-expressed during adult life in the event of skeletal muscle injury/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 MyHCs.

To study the expression dynamics of MyHCs during development, we isolated wild type (C57Bl/6) mouse embryos from pregnant females at 24 hour intervals, starting at Embryonic Day (E) 9.5 and processed the embryos in two ways. Half of the embryos were fixed in 4% Paraformaldehyde, embedded in Optimal Cutting Media (OCT) and frozen for cryo-sectioning. Simultaneously, the other set of embryos were directly frozen for RNA extraction for quantifying gene expression by quantitative Polymerase Chain Reaction (qPCR). The embryos for cryo-sectioning were sectioned using a cryo-microtome and the sections collected on glass slides. Antibodies specific to MyHC-embryonic, -perinatal and -slow were used to detect these proteins on adjacent sections from the same embryo by immunofluorescence, and were imaged using a confocal microscope. The embryos for qPCR were homogenized and total RNA extracted from them, from which complementary DNA (cDNA) was synthesized using which qPCR was performed with primers specific to each MyHC isoform. Using these approaches, we were able to study the expression dynamics of MyHC protein and transcripts during the course of embryonic development. At the protein level, we 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 might play crucial roles during these myogenic phases. The transcript expression pattern did not match the protein expression, with MyHC-embryonic and -perinatal having single peaks of expression at E15.5 and E17.5 respectively, whereas MyHC-slow transcripts were expressed at relatively low levels throughout embryonic development. This suggests that post-transcriptional modifications might be important in regulating the levels of MyHC-embryonic, -perinatal and -slow during vertebrate embryonic development.

To study the function of MyHCs and MyHC-embryonic in particular, we generated a conditional targeted allele for MyHC-embryonic (MyHC-emb$^{fl/+}$) in mouse, the first MyHC to be expressed during development. Using this allele, a knockout for MyHC-embryonic was generated (MyHC-emb$^{\Delta/+}$), by crossing floxed mice (MyHC-emb$^{fl/fl}$) to HPRT$^{Cre/+}$ mice. Mice homozygous for the knockout allele (MyHC-emb$^{\Delta/\Delta}$) were generated by crossing heterozygous parents and we found that the frequency of homozygous mutant progeny was less than half that of the expected Mendelian ratio, suggesting that the MyHC-emb$^{\Delta/\Delta}$ genotype is semi-lethal *in utero*. We also verified that the MyHC-emb$^{\Delta/\Delta}$ allele is null for MyHC-embryonic, by immunofluorescence and qPCR. We isolated individual muscles from MyHC-embryonic knockout and control Postnatal Day 0 (P0) pups and quantified the transcript levels of other MyHCs by qPCR, to verify whether loss of MyHC-embryonic leads to a compensatory up or down regulation of other MyHCs. Interestingly, we find that

the levels of MyHC-IIb, a mature fast MyHC isoform are down regulated and MyHC-IIa, another mature fast MyHC isoform are up regulated in the MyHC-embryonic knockout mice compared to controls, suggesting that loss of MyHC-embryonic indeed has compensatory effects on other MyHCs.

We are in the process of standardizing the conditions for optimal siRNA mediated knockdown of MyHC-embryonic in C2C12 myogenic cells in vitro, so as to study the role of MyHC-embryonic during myogenesis.

We are investigating the expression dynamics of the Transducin-Like Enhancer of Split (TLE) family of corepressors 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, we are also trying to decipher the signals underlying the regulation of the c-MET proto-oncogene in a tumor type called rhabdomyosarcoma (RMS). 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. We aim to use RMS cell lines derived from patients to investigate how Met signaling is regulated in this cancer.

Future plans

Extending our expression analysis for MyHCs performed during development, we will investigate the expression of all MyHCs (including the developmental MyHC-embryonic and -perinatal, the adult MyHC-IIa, IId and IIb as well as MyHC-slow which is expressed during both stages) during P0 to P10 which is the perinatal phase of myogenesis. We will also study whether the adult MyHC-IIa, IId and IIb are expressed during developmental stages. Further, we will decipher the expression dynamics of MyHCs during C2C12 myogenesis. Another interesting aspect would be to identify the regulatory elements of MyHC-embryonic that facilitates it to be expressed in a highly dynamic fashion. We will study this first using C2C12 cells and then consider generating transgenic animals to verify those results.

As part of the functional characterization of MyHC-embryonic knockout animals, we will investigate whether lack of MyHC-embryonic leads to aberrant developmental myogenic differentiation. We will also initiate genetic crosses to generate animals with the correct genotype to perform conditional deletion of MyHC-embryonic during development. In addition, siRNA mediated MyHC-embryonic knockdown will be performed on C2C12 myogenic cells to understand the role of MyHC-embryonic on in vitro myogenic differentiation. We will also expand these studies to include additional MyHCs, MyHC-perinatal and -slow, which are also developmental MyHCs similar to MyHC-embryonic.

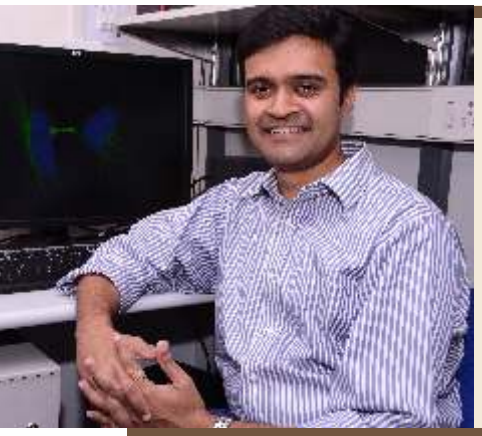
Our preliminary studies indicate that the TLE family of co-repressor genes are differentially expressed during myogenic differentiation and might be potential candidates that regulate differentiation. We will investigate this in greater detail by quantifying transcript and protein expression of the TLE genes by qPCR and western blots respectively using isoform specific primers or antibodies on C2C12 myogenic cell

samples undergoing differentiation. Once the expression dynamics is ascertained, siRNA mediated gene knockdown studies will be initiated to study gene function of specific TLE isoforms during myogenic differentiation.

We will also initiate studies on rhabdomyosarcoma (RMS) cell lines derived from patients. First, we will verify whether c-MET levels are dysregulated in these tumor cell lines. Subsequently, we will address the mechanism behind this dysregulation, specifically focusing on pathways causing ubiqutin-mediated degradation of c-MET, as well as protein interactions that stabilize c-MET.

Publications

1. Keefe AC, Lawson JA, Flygare SD, Fox ZD, Colasanto MP, Mathew SJ, Yandell M and Kardon G (2015) Muscle stem cells contribute to myofibres in sedentary adult mice. ***Nature Commun*** 14:7087.
2. Agarwal M, Kumar P and Mathew SJ (2015) The Groucho/Transducin-like enhancer of split protein family in animal development. ***IUBMB Life*** 67:472.



Mechanisms of Cell Division and Cellular Dynamics

Sivaram V. S. Mylavarapu

Young Investigator
Megha Kumar

Postdoctoral Fellow
Pushpa Kumari

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

Research Theme

Our research group studies the molecular regulation of cellular dynamics. At present we are examining the molecular underpinnings of cell division and intercellular communication, two vital and highly dynamic cellular processes.

Objectives

We wish to uncover the molecular mechanisms of mitotic regulation by the Light Intermediate Chain (LIC) subunits of cytoplasmic dynein. In other projects, we wish 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 third project, we aim to elucidate the mechanistic bases for biogenesis and function of novel modes of intercellular communication. The broad 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.

Progress

Cytoplasmic dynein performs a plethora of functions during mitosis. Our quest to understand the mechanistic roles performed by the LIC subunits of dynein during mitosis led us to uncover novel pleiotropic functions for LIC2 during metaphase. We have elucidated key mechanisms governing the metaphase to anaphase transition by the Light Intermediate Chain (LIC) homologues LIC1 and LIC2 of the molecular motor dynein, using mammalian cell lines and zebrafish embryos as model systems. During studies to dissect the mechanisms by which LIC1 and LIC2 achieve these distinct mitotic functions, we discovered a novel role for LIC2 in enabling the metaphase to anaphase transition, much like LIC1. This effect was however functionally independent of LIC1, suggesting distinct mechanisms of action at metaphase. Quantitative cell biological analyses revealed that LIC2, as part of the dynein complex, specifically removes key subsets of the spindle assembly checkpoint from metaphase kinetochores, thus enabling checkpoint silencing and facilitating metaphase to anaphase progression. Further analyses illuminated other critical roles for LIC2 in spindle maintenance and integrity. LIC2 was found responsible for chromosome congression to the cell equator in metaphase, for regulating spindle length, as well as for maintaining proper orientation of the mitotic spindle angle with respect to the substratum by enabling proper astral microtubule nucleation. In addition, LIC2 is required for properly focusing the spindle poles. LIC2

Scientific Reports

achieves these mitotic spindle functions through interactions with the nuclear protein NUMA, which the lab has established biochemically. Interestingly, LIC1 retains a minor ability to perform several of the LIC2-mediated functions described above, suggesting that LIC2 is the dominant mitotic homologue, except in spindle pole focusing. The mechanistic basis for this uneven distribution of mitotic functions between the LICs is still unclear. We have 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. The lab has communicated this study for publication.

In another project, we have been probing the molecular mechanisms of biogenesis and function of long-range intercellular cytoplasmic conduits (hereafter called nanoconduits or NCs). Cell-to-cell communication plays an important role in physiological processes of multi-cellular organisms. NCs are tubular structures consisting of thin, membrane bridges of diameter in nanometers that mediate membrane continuity between mammalian cells over long distances. The major cytoskeletal components that support NC formation are F-actin fibres, but some NCs containing microtubules have also been reported. Different classes of NCs range in diameter from about 50 nm to 700 nm and often connect cells that are several hundred m apart. NCs have been implicated in a wide spectrum of crucial cellular functions involving intracellular transfer of material: these include transfer of calcium signals, dendritic cell/T cell interactions, transfer of mitochondria from cardiomyocytes to cardiac stem cells, HIV viral transfer, prion transfer and bacterial surfing between cells, skin melanocytes and keratinocytes (melanin transfer), neurons with astrocytes neuroglial cells, and for aiding embryogenesis and development in the absence of morphogen gradients. Despite their demonstrated implications in health and disease, there is very little mechanistic knowledge of the biogenesis and function of NCs.

Recently, the protein NCTAP was reported essential for NC formation. While this is the first step towards understanding molecular regulation of NC biogenesis, there are still no reliable and exclusive biochemical markers for NCs. Moreover, it is unknown which other cellular proteins are involved in NC formation and/ or function. We aim to elucidate the molecular events controlling NC biogenesis and function. We hypothesize that the interaction of NCTAP with its protein interactome promotes NC formation and/ or function. Dissection of these interaction networks and rational inhibition of relevant interactions would illuminate the molecular pathway(s) responsible for NC biogenesis and function.

NCTAP was cloned into an MF (mutifunctional) tagged mammalian expression vector incorporating affinity tags, His8, SBP (Streptavidin Binding Peptide), and FlAG. Stably expressing NCTAP cell lines were generated after transfection of NCTAP-MF recombinant vector into the U2OS (human osteosarcoma) cell line. Similarly empty MF cell lines were generated for control experiments. The function of stably expressing NCTAP was tested in terms of NC formation. After confirming the function of NCTAP in terms of NC formation,

stably expressing cell lines were lysed in cryogenic conditions and cell lysates were subjected to affinity (streptavidin) purification. The eluate of affinity purification was subjected to mass spectrometry (MS) for the identification of the NCTAP interactome. MS results were analyzed by ProteinPilot (which includes both Paragon and Mascot search engines) software with desired parameters. Three biological replicates were performed from both NCTAP-MF and empty MF cell lines independently. Hits common to NCTAP-MF and MF alone were not considered further. Total 33 hits of NCTAP interactome were obtained by Mascot, whereas the Paragon search engine obtained 6 hits. Both search engines had three common hits. Six interacting proteins obtained by Paragon were first considered for characterization, since validation of 33 hits obtained by Mascot is practically challenging. The second part of the eluate of affinity purification was analyzed by Western blotting for biochemical validation of the proteomic hits obtained. Initial Western blot results have confirmed the specific interaction of NCTAP with 3 of the identified proteins, with no interaction observed in the affinity eluate of the empty-MF vector control cell line. These results are being validated by pairwise immunoprecipitation for confirming the interactome. We are also functionally testing whether these proteins are important for NC formation and function.

We biochemically and biophysically characterized the NCTAP protein. We cloned mammalian NCTAP into a His6-tagged bacterial expression vector and expressed NCTAP in bacterial expression strains. We used nickel affinity purification followed by ion exchange and gel exclusion chromatography to purify the protein to near homogeneity. The purified NCTAP protein eluted on gel exclusion columns at the approximate molecular weight of a dimer. We probed mammalian cell lysates to determine whether there was a dimeric pool of NCTAP in the cytoplasm as well. Fractionation of cryogenic mammalian cell lysates on gel exclusion followed by immunoblot analysis of all eluted fractions for NCTAP showed that there is a significant pool of NCTAP that elutes at the dimeric position, in addition to a pool at a larger molecular weight. These pools perhaps represent free dimer and NCTAP complexes. Secondary structure characterization of the purified NCTAP protein on circular dichroism showed that it is a largely helical protein with typical double minima in the spectrum at 222 nm and 208 nm. We also subjected purified NCTAP to limited proteolysis with two different proteases and identified common, independently folded structural domains that were resistant to proteolysis. We have characterized these domains by mass spectrometry and N-terminal sequencing and mapped them within the sequence of NCTAP. These and other domains of NCTAP have been designed, cloned and expressed in bacteria for further purification and biophysical characterization, which is ongoing work.

We have also characterized the NCs by high-resolution optical microscopy to visualize their inner organization. We observed that the NCs range from 200 – 700 nm in diameter, with lengths in the range of several tens of micrometers. Interestingly, NCTAP is localized to discrete regions in the NCs at periodic intervals only in cells expressing the NCTAP-MF construct, but not in the empty vector construct. We have also measured the average dimensions and periodicity of these localized structures. We are presently in the process of confirming this localization by immunofluorescence analysis and also testing whether the NCTAP interactome colocalizes with NCTAP at these regions.

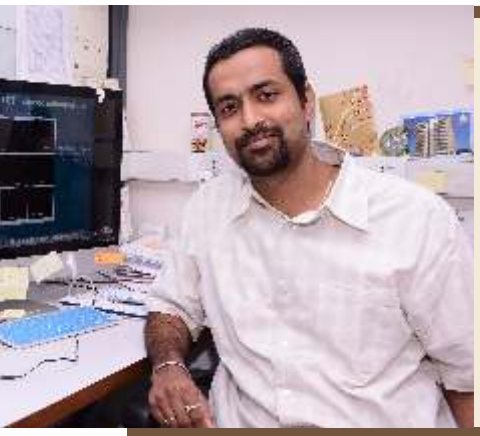
Future directions

We are probing other mechanistic questions regarding the roles of LIC1 and LIC2 in mitosis and the detailed and distinct developmental consequences of perturbing the

function of LICs in metazoan embryogenesis. Answers to these questions will give us a holistic understanding of the molecular mechanisms by which dynein LICs regulate mitotic progression. High-confidence hits from the proteomic screen for the NCTAP interactome will be validated biochemically (pairwise immunoprecipitation using purified proteins), by high-resolution optical microscopy (fluorescence colocalization) and functionally using specific protein depletion by small RNA treatment. Selected protein interacting partners will be characterized biophysically and/or biochemically and functionally by overexpression in mammalian cell lines.

Publications

1. Kumar M, Pushpa K and Mylavarapu SVS (2015) Splitting the Cell, Building the Organism: Mechanisms of Cell Division in Metazoan Embryos. ***IUBMB Life*** 67: 575.



Studies on biology of infectious and idiopathic inflammation of the gut

Chittur V. Srikanth

Young Investigator
Smriti Verma

Postdoctoral Fellow
Mukesh Singh

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

Project Staff
Navneet Kaur

Research Theme

Our laboratory works on several very important diseases of the Gastrointestinal (GI) tract including autoimmune diseases of gut and illnesses arising from pathogenic bacteria. Using state of the art tools including animal models and clinical samples we try to understand complex molecular mechanisms underlying these diseases.

Objectives

- Identify novel bacterial virulence proteins that mediate intestinal inflammatory pathways
- Investigations host molecular pathways that get affected during infections To test if the identified pathways are also operational during states of autoimmune disorders and other illnesses of the gut

Progress

The GI tract is often exposed to chemical agents and pathogens that enter our body through contaminated food or water. Occasionally these agents breach the mucosal barrier, leading to inflammation and disease. 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, gastroenteritis include fever, diarrhoea, vomiting, abdominal cramps and often lead to hospitalizations. We have been investigating using model pathogen ST to unearth novel mechanisms that are key in the states of inflammation and disease. We earlier identified that host SUMOylation, a post translational modification pathway, was significantly altered by ST during infection. The SUMO machinery, similar to the ubiquitylation machinery, utilizes three enzymes that include an activating enzyme E1, a conjugating enzyme E2 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.

We observed ST significantly alter host SUMOylation in cell culture and mouse model, a process that was dynamic over the course of infection. The SUMO alteration required live ST with functional Type Three Secretion System (TTSS). The ST mediated SUMO alteration was accompanied by a concomitant down-regulation of the E2 SUMO enzyme, Ubc9 and the E3 enzyme, Pias1. We were also able to demonstrate that SUMOylation downregulation carried out by ST was critical for it to control the inflammatory pathways of

Scientific Reports

the host. A qPCR analysis of inflammatory genes, in host cells that were upregulated for their SUMOylation machinery and infected with ST, displayed a dramatic dysregulation of inflammatory genes.

In the current year (2014-2015) we have furthered our understanding with regard to the mechanisms. In order to identify the SUMO-conjugated proteome (SUMOylome) that undergoes alteration during ST infection we carried an unbiased proteome screen using high resolution Mass-spectrometry. We utilized a method that relied on the capacity of RNF4, a ring-finger domain containing E3 ubiquitin ligase, to bind to SUMO-2/3 conjugated proteins. Presence of multiple SIMs (SUMO interacting motifs) in RNF4 enables it to interact with SUMOylated proteins. The wild type RNF4 encoding gene was cloned as a GST tagged version (called as wt- RNF4-GST in *E. coli*). The tagged protein was expressed and purified as a GST-conjugated protein. Separately similar to the wt-RNF4, we purified a mutant GST version of RNF-4 (now onwards called as smut-RNF4-GST) devoid of SIMs. The entire clone Wt-RNF4-GST and smut-RNF4-GST was sequenced to ensure the correctness of the construct. The purified smut-RNF4, which was incapable of binding to SUMO conjugated proteins, was used in the study to eliminate non-specific binders. The purified Wt-RNF4-GST is designed to act as an affinity purification system specifically for SUMOylated proteins. These, along with the smut-RNF4-GST bound to glutathione beads were utilized to specifically isolate SUMOylated proteins from cell lysates of ST infected and control cells. The affinity purified samples were concentrated, de-salted, processed for ESI-Mass-spectrometry (AB SciEx 5600). The spectra of the complex proteome obtained were run through the software ABSciEX (version ANALYST TF1.5.1) to individually identify the parent protein. The offline software used was Protein Pilot (MASSCOT and PARAGON). We identified 165 proteins in uninfected sample and 98 proteins in ST infected sample. The obtained proteome that was unique to the Wt-RNF4 affinity purification, but not with the smut-RNF4, were identified as true SUMO-conjugated proteome. Our analysis revealed several interesting candidates that appeared to be SUMOylated or de-SUMOylated during ST infection. For further validation we examined these samples through a SUMO motif and SIM identification software (sumosp.biocuckoo.org/online.php). This quality control enabled us to eliminate proteins unrelated to SUMO. We picked only those candidates that harbour either a SIM, or a SUMO motif or both. These identified candidates included dactin binding proteins (such as Ezrin, profiling-1), histone modifiers, nuclear matrix components (such as Lamin-A/c), endocytic vesicular transport proteins (Rab33B, rab6a, Rab7a, Gnai3 etc), translation regulator, enzyme regulatory proteins, antioxidant proteins. Notably several were from the intracellular vesicular transport system. This was interesting since the intracellular life of ST immensely relies on the vesicular trafficking of the host cell. ST is known to interact closely with the vesicular pathway mediators such has the Rho GTPases and the Rab family proteins. Within host cells, ST survives in a membrane bound compartment called Salmonella containing vacuole (SCV). The SCVs extensively interact with the endocytic pathway that is solely dependent on the vesicular transport of the cell. These interactions result in acidification of the SCV, which in turn lead to enrichment of the lysosomal membrane glycoproteins in the SCV membrane. Since our infection specific altered SUMOylome has several members from the vesicular transport system we investigated this aspect in greater detail.

Our candidate list has proteins such as RNF4, Lamin-a/c which are known to be SUMOylated though not particularly upon ST infection. On the other hand we have proteins such as Ezrin, Profilin, Rabs, TRIM, and NPM1 that are very important from the relevance of ST but are not known to be SUMOylated. It is noteworthy that several of the

identified candidates have multiple SUMO motifs and SIMs implying a possible connection with SUMO machinery. Experiments to confirm SUMOylation of some of these candidates is underway.

In addition our efforts are also focused to get a better read-out in the mass-spectrometry. To achieve this end we are currently carrying out ESI-MS/MS by using isobaric labelling methods (iTRAQ labelling). We are in the process of complementing our data set by a separate MALDI-TOF (AB Sciex 5800) analysis of our samples of both SUMO2/3 and SUMO1 modified proteome. This additional experimentation will expand our understanding of infection specific altered SUMOylome.

To address this aspect from another angle we investigated the intracellular life ST in the host. We had earlier observed that SUMOylation regulated the post-entry survival of ST and not the entry process per se. Thus this observation and our proteome analysis suggest that the life of ST in SCV may be under SUMO regulation. Several lysosomal proteins usually decorate the SCVs at different phases of infection. LAMP1 is one such protein that is recruited during late stages of infection that is a crucial determinant of the fate of SCVs (Fig. 1A), was used as a marker for SCVs. SUMO perturbed and control cells were infected with mCherry labeled ST (hereafter mST) and intracellular localization of LAMP1 were followed by confocal microscopy. In control cells, LAMP1 stained SCVs and SIFs (Salmonella induced filaments) were distinctly visible (Fig 1B left panel). Contrary to this, in SUMO1 upregulated cells, there were fewer bacteria in the LAMP1 stained compartment in concordance with our previous results. These SCVs were significantly compromised for SIFs (Fig. 1B right panel). We inferred from the above data that, the compromised intracellular survival of ST in SUMO upregulated cells observed previously by various experiments are resultant of unstable SCVs that are deprived of SIFs.

We investigated Rab7, a GTP binding protein of the Rab family, for its connection with the SUMO machinery. In untreated epithelial cells, in line with the earlier reports, ST infection led to an increase in the expression of Rab7 (Fig. 1C). In contrary to this, in cells that were transfected with SUMO1 expressing plasmids so as to upregulate the overall SUMOylation, ST infection did not result in Rab7 upregulation. Rab7 recruitment to SCV and its interaction with RILP (Rab7 interacting lysosomal protein) is critical for the SCV stability. We therefore examined interaction of Rab7-RILP in SUMO upregulated infected cells. In Co-immunoprecipitation experiments, unlike the control cells where ST infection led to increase in Rab7-RILP interaction, the SUMO1 upregulated cells displayed a decrease in RIL-rab7 interaction. Specificity of this interaction was confirmed by control IgG antibodies (Fig. 1D). A reduced Rab7 localization in SUMO1 overexpressing cells was also seen in imaging experiments (data not shown).

Our next important objective was to investigate the mechanism by which ST is able to alter the host SUMoylation. Literature available on Ubc-9 downregulation suggests a role for microRNAs particularly those belonging to members of the miR30 family in certain cancers (Wu et al., 2009). The microRNAs (miRNAs) are small non-coding RNAs, known to play crucial role in eukaryotic gene regulation that are transcribed in the nucleus precursors that get processing by Drosha and Dicer into mature miRNA which act as regulator of transcription and translation. For the current study we chose two miRNAs – miR30c and miR30e. ST infected and control samples were quantified for the presence of miR30c and miR30e by qPCR to check if they have any role in ST induced Ubc-9 downregulation. ST infection resulted in upregulation of mature forms of miR30c and miR30e in a time course of infection. The miR30c and miR30e levels were upregulated from as early as 30 minutes following infection in comparison to control cells. For miR30e,

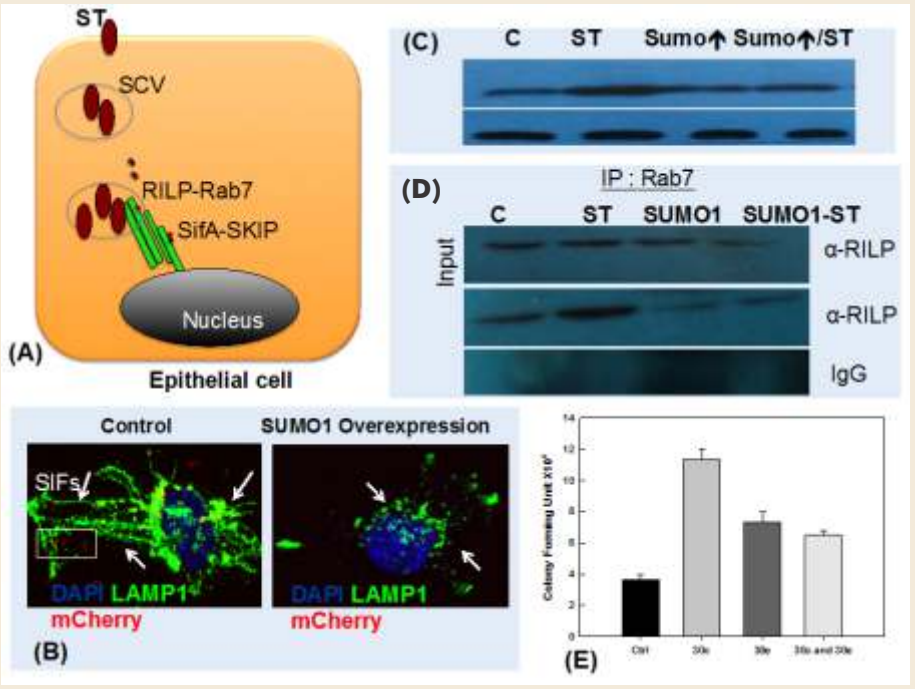


Figure 1. SUMOylation plays a key role in ST infection. A) Schematic representation of various stages of ST infection. B) SUMOylation overexpression blocks SIF formation as revealed by confocal microscopy of control and SUMO1 overexpressing HeLA cells infected with ST. C) Rab7 immunoblots of control and SUMO overexpressed cells either infected with ST or kept untreated. D) Immunoprecipitation of lysates with Rab7 antibodies of control and SUMO overexpressed cells either infected with ST or kept untreated, followed by immunoblotting with RILP antibodies or control IgG. E) Gentamycin protection assays were performed on HCT8 cells that were transfectedwith the indicated miRNA mimics followed by ST infection. We have recently published a portion of this work [Verma et al. (2015) Mol Cell Biol 35:2932].

but not miR30c, this upregulation was absent when the cells were infected with a SPI1 mutant (DSPI1) indicating that the upregulation was specific to wildtype bacteria requiring entry as was seen in case of the SUMO alteration (Data not shown). To validate the findings we transfected the cells with miRNA mimics specific for miR30c and miR30e and examined the expression levels of Ubc9. We observed that these mimics were able to downregulate the expression of Ubc9 almost to the same effect as seen during ST infection. On the contrary in presence of the inhibitors the ST mediated Ubc-9 downregulation was nullified. These data suggested that miR30c and miR30e were sufficient to downregulate Ubc9. To test if the presence of the miR30c and miR30e could actually alter the intracellular survival of ST we examined the cfu by Gentamycin protection assay (GPA) in cells that were transfected with the miRNA mimics and inhibitors followed by ST infection. In the presence of miR30c and miR30e mimics, we observed more than two-fold increase in the cfu of ST at 7hrs post infection while the presence of inhibitors abrogated this effect (Fig.1E). These experiments corroborated that ST mediates Ubc9 downregulation by upregulating members of miR30 family of miRNAs.

Together our data establishes the importance of SUMOylation machinery as an integral mechanism operational during ST infection and inflammation (Fig. 2).

Scientific Reports

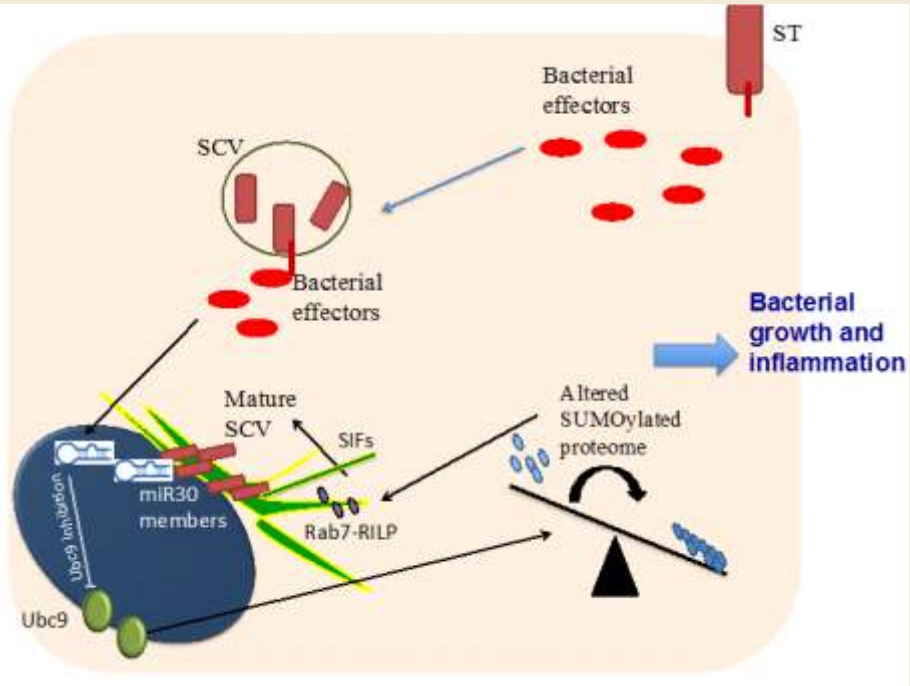


Figure 2: Schematic representation of ST mediated SUMO alteration and its importance in SCV maintenance, bacterial replication and induced inflammation.

Future plans

- Investigations of molecular mechanisms of ST mediated SUMO alterations
- Examining the possible role of SUMO pathway in IBD and colon cancer in patient samples and in animal model system

Publications

1. Verma S, Mohapatra G, Ahmed SM, Rana S, Jain S, Khalsa JK and Srikanth CV (2015) Salmonella engages host microRNAs to modulate SUMOylation: a new arsenal for intracellular survival. ***Mol Cell Biol*** 35:2932.
2. Hallstrom KN, Srikanth CV, Agbor TA, Dumont CM, Peters KN, Paraoan L, Casanova JE, Boll EJ and McCormick BA (2015) PERP, a host tetraspanning membrane protein, is required for Salmonella-induced inflammation. ***Cell Microbio*** 17:843.
3. Verma S and Srikanth CV (2015) Understanding the complexities of Salmonella-host crosstalk as revealed by *in vivo* model organisms. ***IUBMB Life*** 67:482.

Scientific Reports

Post-translational protein modification: involvement in cellular processes and disease regulation

Tushar Kanti Maiti

Collaborators
Nihar Ranjan Jana (NBRC)
Neel Sarovar Bhavesh (ICGEB)

Young Investigator
Sushmita Bhattacharya (till July 2015)

Postdoctoral Fellow
Deepak Jangir
Amit Kumar Dey
Bhoj Kumar

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

Project Staff
Abhishek Kumar Singh
Neha Sharma
Neelam Antil

Research theme

The major interest of our research group is to understand how posttranslational modifications of protein regulate diverse cellular signaling events and their mechanism of disease regulation.

Objectives

Protein post translational 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.

- One of the important aspects of our research 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. We are investigating 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 lead to diseases like Parkinson, Alzheimer, ataxia, heart disease and different types of cancer. Our aims are to understand the possible molecular mechanism underlying these diseases.
- Another aspect of our lab is to understand how redox modification particularly Cys-Nitrosylation regulates diverse cellular processes including disease outcome from neurodegenerative disease to microbial infection.

Progress

Since last three decades considerable progress occurred in genome sequencing field that reveals genomic landscape of cancer. Advancement of genomic studies showed 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. In recent years, BRCA1 associated protein 1 (BAP1), a nuclear deubiquitinating enzyme that has emerged as an important tumor suppressor protein, undergoes frequent mutations in different types of tumor and appears as one of the driver genes in cancer types like uveal melanoma, mesothelioma, renal cell carcinoma, cholangiocarcinoma and melanocytic tumors. However the role of mutation and oncogenic gain of function of BAP1 are poorly understood. Impairment of enzymatic activity and nuclear localization of BAP1 induce

Scientific Reports

abnormal cell proliferation. Here, we investigated cellular localization, enzymatic activity and structural changes due to the missense mutation in the catalytic domain of BAP1, which are prevalent in different types of cancers. We have shown that these mutations triggered cytoplasmic/perinuclear accumulation in BAP1 deficient cells, which has been observed in many proteins that undergo aggregation in cellular condition. Amyloidogenic activity of mutant BAP1 was revealed from its reactivity towards anti oligomeric antibody in HEK293T cells. We have also noted structural destabilization in the catalytic domain mutants, which eventually produced beta amyloid structure as indicated in atomic force microscopy study. The cancer associated mutant up-regulates heat shock response and activates transcription of genes normally co-repressed by BAP1. Overall, our results unambiguously demonstrate that structural destabilization and subsequent aggregation abrogate its cellular mechanism leading to adverse outcome.

Regulation of Parkinson disease (PD) pathology by Ubiquitin C-terminal hydrolase-1 (UCHL1) has been elucidated however molecular mechanism its involvement is not still clear. It is a deubiquitinating enzyme, which abundantly expressed in neurons and constitutes almost 1-2% of total soluble brain proteins. It has been shown that one polymorphism in *UCHL*1 gene; I93M induces whereas S18Y protects PD. Several attempts have been made in correlating polymorphism of UCHL1 and their correlation with protein stability, enzymatic property, understanding of structural variability and preferential posttranslational modifications however the clear understanding of disease pathology is still elusive. Parkinson's disease is a neurodegenerative movement disorder characterized by degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) of midbrain. Neuronal cell death is accompanied by the accumulation of different types of poorly degraded proteins and the formation of proteinaceous inclusions called Lewy bodiesin dopaminergic neurons. Here we have strived correlating how redox stress regulates UCHL1 function and induces PD phenotype in mouse model. Neurodegeneration is an age related disorder, which is induced by exposure to environmental toxins. Environmental factors stimulate mitochondrial dysfunction and alteration of Nitric oxide synthase machinery leading to increase in NO production. Nitrosative stress is believed to contribute largely in pathogenesis of sporadic PD, but the exact molecular basis remains unclear.

The mass spectrometry based Cys-NO identification of GSNO induced cysteine nitrosylation in SHSY-5Y cells demonstrated that UCHL1 is one of the proteins that undergoes nitrosylation in nitrosative stress condition. This finding prompted us to explore how cysteine nitrosylation regulates UCHL1 function. In this context we asked a question if UCHL1 nitrosylation is associated with Parkinson disease? To address this question we have established both *in vitro* and cell culture based nitrosylation assay. We have shown that UCHL1 is S-nitrosylated at three critical residues Cys 90, Cys 152 and Cys 220. Nitrosylation of Cys 152 residue is observed by the treatment of GSNO with UCHL1 in equimolar ratio however 10 molar excess of GSNO treatment leads to the modification Cys90 and Cys220 modification in addition of Cys152. It has been demonstrated earlier that Cys 152 is the residue that undergoes prostaglandin modification and carbonyl modification. The prostaglandin modification induces structural instability. Our enzymatic activity assay, circular dichroism study, gel filtration and NMR experiment demonstrated that NO-induced S-nitrosylation of UCHL1 decreases its enzymatic activity, structural destabilization which ultimately lead to the aggregation of UCHL1.

Our finding clearly demonstrated that nitrosylation of UCHL1 and subsequent reduction of catalytic function and structural destabilization is one of the major components that induce PD pathogenesis and this finding may thus bridge a molecular link between nitrosative stress and accumulation of UCHL1 in sporadic PD.

Future plans

We have shown for the first time that BAP1 mutation induces structural destabilization leading to the formation of beta-amyloid aggregate. However how beta-amyloid aggregate of BAP1 is linked to cancer pathology is still not clear. Loss of BAP1 function due to mutation is associated with dysregulation of BAP1 cellular function however gain of toxic function due to protein aggregation needs to be investigated. There are a few examples where tumor suppressor protein p53 showed beta aggregation and induces gain of toxic function leading to cancer. In fact, prion and protein-only inheritance in cancer is recently appreciated. Amyloidogenicity of BAP1 will inevitably strengthen the prion hypothesis in cancer biology; however a detailed molecular study is necessary to understand its aggregation mechanism. We have hypothesized that oligomerization of mutant BAP1 inhibits PR-DUB complex formation and deubiquitination of histone 2A due to loss of its catalytic activity and impairment of nuclear transport that culminates into enhancement of oncogenic activity thereby explaining the effect of missense mutations in BAP1. Finally, correlating BAP1 mutation and cancer outcome at molecular level will help us to understand tumor biology and potential therapeutic development to combat cancer in general.

Cysteine nitrosylation of UCHL1 and correlation with Parkinson diseases pathology will be tested in rotenone induced mouse model. Rotenone is a pesticide that disrupts the mitochondrial function and induces redox stress. It is considered of being one of the reproducible mouse models for PD. It has been shown that rotenone treatment to SHSY-5Y cells induces the nitric oxide. We would like to investigate the nitrosylation status of UCHL1 in rotenone treated mouse.

We also propose to investigate how nitrosylation of UCHL1 induces its cellular pathway. We will also explore whether nitrosylation of UCHL1 can induce the aggregation of alpha synuclein, which is the major constituent of Lewy bodies. Further we will assess the cross talk PINK-DJ1-UCHL1 in the context of nitrosylation. Nitric oxide is one of the most versatile players that regulate our immune system and dysregulation leads to many diseases like cardiovascular, diabetes, neurodegenerative and cancer. Oxidative burst is a phenomenon that leads to nitric oxide generation in micromolar amount that kills invading pathogens. Investigating the ability of different intracellular pathogens to withstand the toxic effects of NO will give a deeper insight towards pathogen resistance. Also NO-mediated immunity in the host defence can be boosted to enhance host resistance to pathogenic diseases.

Publications

1. Hanpude P, Bhattacharya S, Dey AK and Maiti TK (2015) Deubiquitinating enzymes in cellular signaling and disease regulation. ***IUBMB Life*** 67:544.

Scientific Reports

Structural Biology of Host-Microbial Interactions in Health and Diseases

Vengadesan Krishnan

Postdoctoral Fellow
Arjun Kumar Mishra
Shivendra Singh

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

Research Theme

Understanding the structural basis of host-microbial interactions in health and diseases is our broad theme. We are currently focusing on cell surface proteinaceous assemblies like pili from beneficial and pathogenic bacteria, and aiming to elucidate common and specific bacterial strategies in probiosis and pathogenesis.

Objectives

- Investigating pilus architecture, assembly and adhesion process in beneficial and pathogenic bacteria.
- Understanding bacterial pilus-mediated adhesion strategies in initiating host interactions.

Progress

Bacteria often assemble hair-like appendages known as pili or fimbria on their cell surfaces for different purposes such as adherence, twitching motility, conjugation, immunomodulation, biofilm formation, and electron transfer. The pili are known as virulence factors for decades in pathogenic bacteria owing to their key role in the initiation of adhesion for pathogenesis. They are also ideal vaccine candidates because of their immunogenic properties and exposure on the cell surface. The pili are typically made of building blocks called pilins or fimbrilins. Multiple copies of major pilins form the pilus shaft, while a few copies of ancillary pilins decorate it for adhesion and other functions. Because of the key role in adhesion and the exposure on the cell surface, targeting the pilins-mediated adhesion (e.g. anti-adhesion therapy) is seen as a promising alternative approach for preventing and treating bacterial infections, one that may overcome their ever-increasing repertoires of resistance mechanisms.

Pilins interact with each other non-covalently to form the pilus fiber with the help of associated proteins in Gram-negative bacteria. In contrast, the pilins in Gram-positive bacteria often interact covalently with the help of sortases to assemble pilus fiber. Certain unique structural features present on the pilins distinguish them from one another across different bacterial strains, and these dictate their cellular targets and functions. While the structure of pilins has been extensively studied in Gram-negative pathogenic bacteria, the pilins in Gram-positive pathogenic bacteria have been in focus only during the last decade. Recently, the discovery of pilins in nonpathogenic bacteria, such as *Lactobacillus*

Scientific Reports

rhamnosus GG, has received great attention, though traditionally the attention was on pathogenic bacteria. Currently, we have chosen Lactobacillus *rhamnosus GG* as a model and continued our structural investigation programme on its pilus constituents towards understanding the pilus-mediated adhesion strategies of beneficial bacteria.

In pathogenic bacteria, the genes encoding the pilins and related proteins for pilus biogenesis are commonly located at the same locus called pilus gene cluster or Pathogenicity Island (PI) as it contains genes associated with virulence. Genome of L. *rhamnosus GG* contains two pilus gene clusters named *SpaCBA* and *SpaFED*. The *SpaCBA* pilus cluster consists of genes for a major pilin SpaA, two ancillary pilins SpaB and SpaC, and a pilin-specific sortase SrtC1. The *SpaFED* pilus cluster contains genes for a major pilin SpaD, two ancillary pilins SpaE and SpaF, and a pilin-specific sortase SrtC2.

The major pilin SpaA was purified, and crystallized in monoclinic space group C2 with unit cell parameters a = 227.9 Å, b = 63.2 Å, c = 104.3 Å, β = 95.1°. A native dataset has been collected to 1.9 Å at synchrotron source BM14, ESRF (Grenoble, France). After unsuccessful attempts to solve the structure by conventional molecular replacement (MR), selenium/sulfur single-wavelength anomalous dispersion (SAD) and other heavy atom derivative methods, we used fragmented approach that yielded interpretable electron density map. The fragments of SpaA were crystallized in orthorhombic space group P21212 with unit cell parameters a = 57.08 Å, b = 74.36 Å, and c = 116.8 Å as well as in tetragonal space group I422 with unit-cell parameters a = 100.35 Å, b = 100.35 Å, and c = 57.34 Å. The crystals diffracted to 2 Å resolution at the home-source. Ensemble of models were created for the fragments and used in the calculation of structure solution. Model building and refinement has been completed and structural analysis is being carried out.

SpaD, a major pilin in SpaFED pili has also been purified and crystallized in various conditions (Figure 1). A native dataset has been collected to 3 Å at the synchrotron source. While an attempt to solve the structure by Se-SAD phasing is under progress, the fragmented approach was used similar to SpaA. Native data set for crystals of SpaD fragment have been collected to 2.0 Å at the home source. The halide quick soaking method was used to prepare sodium iodide derivatized crystals, and a derivative dataset has been collected at home source to 2.0 Å resolution. Structure solution was obtained by iodide-SAD phasing. The model building and refinement have been completed, the structural analysis is being performed.

SpaC is a larger ancillary pilin in SpaCBA pili. Surprisingly, this pilus adhesin seems to be decorated along the shaft. Studies have shown that SpaC is a key-binding factor to human intestinal mucus, collagen and intestinal epithelial cells (IECs). SpaC has also been shown to stimulate biofilm development, adhere to the fetal gut and modulate TLR-related gene expression. SpaC has been purified and crystallized in several conditions. Optimization of one of the conditions, yielded petal-shaped single crystals (Figure 2), and a native data has been collected to 2.8 Å at the synchrotron source. Attempt to solve the structure by Se-SAD phasing is under progress.

Figure 1. SpaD Crystal

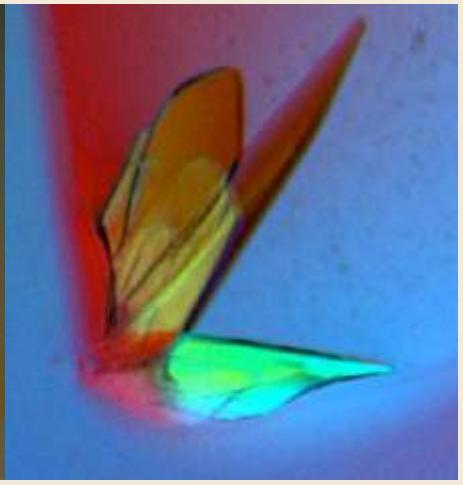


Figure 2. SpaC crystals

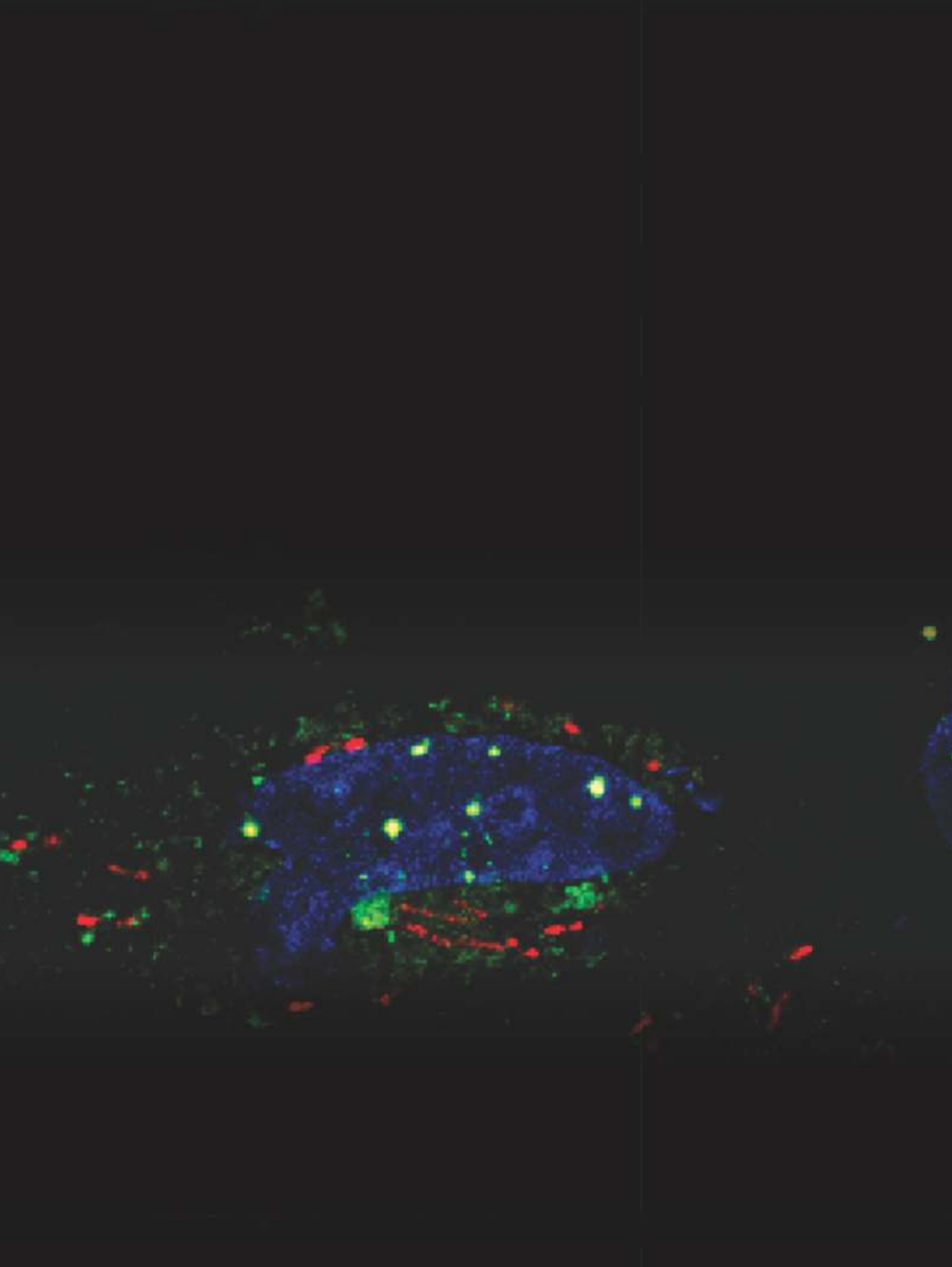
Future plans

Structural and functional investigations of pilus constituents from *L. rhamnosus GG* will be continued in the projected path. Structural analysis will be carried out for major pilins SpaA and SpaD. Structure solution for pilus adhesin SpaC will be attempted by Se-SAD. Purification of pilus components from primary colonizers that mediate adherence to oral tissues and interaction with secondary colonizers for oral biofilm (plaque) development will be attempted towards understanding tissue tropism and the adhesion strategies.

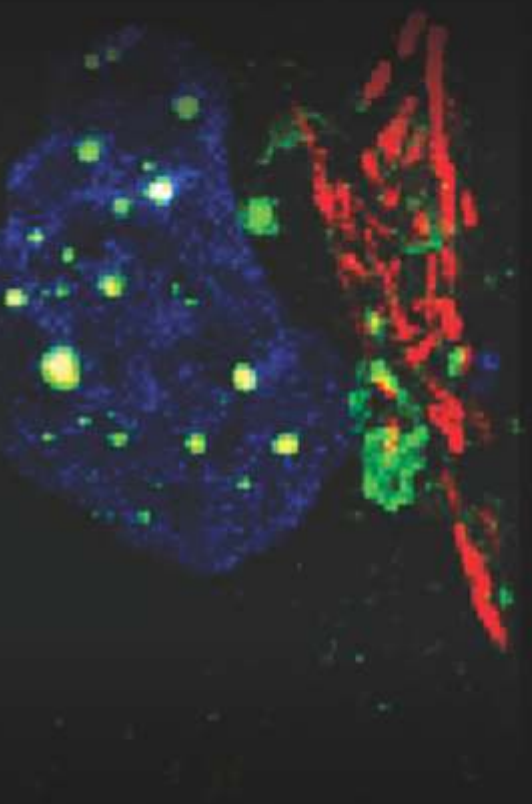
Publications

1. Chaurasia P, von Ossowski I, Palva A and Krishnan V (2015) Purification, crystallization and preliminary X-ray diffraction analysis of SpaD, a backbone-pilin subunit encoded by the fimbrial spaFED operon in *Lactobacillus rhamnosus GG*. ***Acta Cryst*** F71: 103.
2. Krishnan V (2015) Pilins in gram-positive bacteria: A structural perspective. ***IUBMB Life*** 67:533.

Scientific Reports



Academic Activities & Achievements



Education & Training

Multidisciplinary PhD Programme

Multidisciplinary doctoral programme has been instituted for students who have completed Masters 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) to work under the mentorship of the faculty. The fellowship is initially tenable for the duration of three years and is extendable for additional two years after a review. JRFs are recruited once during the academic year. As on the current academic year, 64 students are enrolled as JRF/ SRFs leading to the PhD degree. RCB has entered into an agreement with Manipal University for registration of students.

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 to carry out research in different areas of biotechnology. During the past year, the Young Investigator programme has been augmented and suitable candidates have been recruited. In addition to YIs, 10 post-doctoral fellows are also being mentored by the RCB faculty.

Short-term Training

Post-graduate students have the opportunity to conduct short research projects and/or dissertation work towards partial fulfilment of their degrees. Over 100 students have so far benefitted from this programme.

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

Master's Degree in Medical Science

The Centre is at the early stages of conceptualizing an Integrated Masters' programme in Biotechnology leading to a PhD degree. The overall aim of this course is to foster practical learning and research skills in medical and related graduates to enhance their overall educational experience and enable the development of competent medical researchers. The programme is designed with the objective of providing knowledge in life sciences with emphasis on human biology, clinical and translational research. The programme will derive expertise from within RCB as well as other national institutions including National Institute of Immunology (NII), Translational Health Science and Technology Institute (THSTI), and National Institute of Biomedical Genomics (NIBMG).

Achievements & Academic Activities

Status of RCB Bill

The revised RCB Bill 2015, after inputs from DBT and Department of Legislature, and the Department of Legal Affairs, Ministry of Law & Justice has been drafted. The views from the various stakeholders viz., Ministry of External Affairs, Ministry of Human Resources Development, Ministry of Environment Forest and Climate Change, Ministry of Finance and public were solicited. The RCB Bill incorporating the suggestions and inputs following due processes will be taken up for placing in the forthcoming Lok Sabha session.

Workshops & Study Visits

An advanced workshop on Diabetes was organized by RCB in partnership with the International Union of Biochemistry and Molecular Biology in November 2014. The workshop was attended by select participants from all over India and they were tutored by instructors from different parts of the country. The attendees and instructors also included participants from the South Asian region and other countries.

The Centre also organized an International Workshop-cum-symposium titled "Frontiers of Structural Biology New Advances in X-Ray Diffraction and Cryo - Electron Microscopy" in December 2014. This meeting was conducted in partnership with Indian National Science Academy (INSA) and the International Union of Crystallography. The event was part of the International Year of Crystallography (2014). The conference and workshop were attended by young, mid-career and senior scientists from all over the world.

UNSECO India Cluster Officer and RCB will soon organize a Regional Dialogue on Science & Technology Policy in the context of Biotechnology. In this meeting, eminent scientists, policy makers, and academicians from 6 Asian Countries will participate. This will be a unique event where policy makers, scientists and industry leaders in the respective areas will present country-specific strategies on Science & Technology - especially Biotechnology to achieve sustainable development for all.

Achievements & Academic Activities

Achievements & Academic Activities

Lectures Delivered/ Conferences attended/ Visits abroad

Dr. Avinash Bajaj

1. Delivered an invited lecture entitled "Bile acid amphiphiles targeting cellular membranes for next generation therapeutics" at 39th Annual Indian Biophysical Society meeting held at Jamia Millia Islamia, New Delhi, India, during 14-17 February 2015.
2. Attended and presented poster entitled "Engineering of controlled release nanoparticles for cancer therapy" at Indo-US workshop on nano-engineering in medicine held at All India Institute of Medical Sciences, New Delhi, during 17-19 December 2014.
3. Delivered an invited lecture entitled "Emerging trends in nanotechnology for cancer therapy" at National Conference on Understanding the mechanism and challenges of Complex Diseases organized by Shaheed Rajguru College of Applied Sciences for Women, University of Delhi, during 29-30 December, 2014.
4. Delivered an invited lecture entitled "Metabolic syndrome and onset of cancer: Etiological roles, prevention strategies and nanotechnology based therapeutics" at IUBMB-RCB Advanced School-2014 Diabetes and Metabolic Syndrome Networks, Crosstalks and Interventions, held at Heritage village resort, Manesar Gurgaon, India during 24-29 November 2014.

Dr. Deepak T. Nair

1. Delivered a seminar titled "Protein Expression and Purification" at the "Workshop on Structural Biology: An introduction to Protein Crystallography" held at the NIMHANS, Bangalore during 15-18 April 2015.
2. Delivered an invited lecture titled "Mutagenic and translesion DNA synthesis by DNA Polymerase IV from *Escherichia coli*: twin routes to drug resistance." at the International Congress on Friedreich's Ataxia and DNA structures in Health and Disease held at the All India Institute of Medical Sciences, New Delhi during11-13 April 2015.
3. Delivered a seminar titled "DNA Polymerase IV and Antibiotic Induced Oxidative Stress" at the Bio-Epoch 2015 held at the School of Biotechnology, Jawaharlal Nehru University during10-11 April 2015.
4. Delivered a seminar titled "DNA Polymerase IV and Oxidative Stress" at the 39th Annual Meeting of the Indian Biophysical Society held at Jamia Millia Islamia, New Delhi, India during 14-17 February 2015.
5. Delivered a mentor Seminar titled "Mechanistic Insights into Bacterial and Viral Replication" at the Ramalingaswami Fellows Conclave held at Bhubaneshwar, Orissa during 30 January-1 February 2015.
6. Delivered a seminar titled "A close look at translesion DNA synthesis by DNA polymerase IV from *Escherichia coli*" at the International Symposium cum workshop titled Frontiers in Structural Biology: New Methods in X-ray Diffraction and Cryo-Electron Microscopy held at the Indian National Science Academy, New Delhi during15-17 December 2015.

7. Session Chair at the IUBMB/RCB Advanced School on Diabetes and Metabolic Syndrome: networks, crosstalks and interventions held at the Heritage Village Resort, Manesar, Gurgaon, India, during 24-29 November, 2014.
8. Delivered a seminar titled "Structural basis of mutagenic and translesion DNA synthesis by DNA polymerase IV from *Escherichia coli*" at the 43rd National Seminars in Crystallography held at Central Drug Research Institute, Lucknow during12-14 November 2014.
9. Delivered a seminar titled "Replication of the flaviviral genome: Structure of a pre-initiation state and mechanism of initiation" at the Indo-US conference meeting on Recent advances in Structural Biology and Drug Discovery (RASBDD-IIT-2014) at Indian Institute of Technology - Roorkee during 9-11 October 2014.

Dr. Deepti Jain

1. Delivered an invited lecture titled "High Resolution Crystal Structures of transcription factor AraR in complex with four different DNA sequences reveal distinct modes of binding" at the International Congress on Friedreich's Ataxia and DNA structures in Health and Disease held at All India Institute of Medical Sciences, New Delhi during11-13 April 2015.
2. Delivered an invited lecture titled "Structures of four natural operators bound to AraR reveal distinct binding modes of transcription factor" at the 83rd Annual meeting of the Society of Biological chemists of India at Bhubaneshwar during18 -21 December 2014.
3. Delivered an invited lecture titled "High Resolution structures of four natural operators bound to AraR reveal distinct binding modes of transcription factor" at the INDO-US conference and workshop on Recent advances in Structural Biology and drug discovery at IIT-Roorkee during 9-11 October 2014.

Dr. Dinakar M. Salunke

1. Delivered a lecture entitled "Structural biology of immune recognition" at IIT-Kanpur on 9 September 2015.
2. Visited UK as a member of delegation led by Hon'ble Minister of State for Science & Technology and ES to participate in meetings with UK counterpart during 21-23 May 2015.
3. Delivered an invited lecture entitled "New paradigms in immune recognition" at Young Investigators Meeting-2015, Srinagar, J&K during 28-31 March 2015.
4. Delivered an invited lecture entitled "Structural Bioinformatics of seed proteome" at National level workshop on "Current Trends in Herbal Informatics", RMRC, ICMR, Belgaum on 23 March 2015.
5. Delivered an invited lecture entitled "Biotech Science Cluster" at DBT's Scientific Advisory Committee - Overseas meeting during 25-26 February 2015.
6. Delivered an invited lecture entitled "Regional Centre for Biotechnology: Genesis and importance" at Symposium on "Role of Biotechnology in post-2015 Development Agenda", UNESCO, Paris on 13 February 2015.
7. Delivered an invited lecture entitled "Adjustable locks and flexible keys: New paradigms in antigen recognition" at IUPAC's International Symposium, IISER, Pune during 13-14 January 2015.
8. Delivered an invited lecture entitled "Adjustable locks and flexible keys: New paradigms in antibody specificity" at 83rd Annual meeting of the Society of Biological chemists of India at Bhubaneshwar during18 -21 December 2014.

9. Delivered an invited lecture entitled "Plasticity of antigen recognition in humoral immune response" at the International Symposium cum workshop titled Frontiers in Structural Biology: New Methods in X-ray Diffraction and Cryo-Electron Microscopy held at the Indian National Science Academy, New Delhi during15-17 December 2015.
10. Delivered a lecture entitled "100 years of X-ray crystallography: A historical perspective" at Regional Centre for Biotechnology on 21 October 2014.
11. Delivered an invited lecture entitled "Adjustable locks and flexible keys: plasticity in immune recognition" at South Asian University on 17 October 2014.

Dr. Divya Chandran

1. Attended International Symposium cum workshop titled Frontiers in Structural Biology: New Methods in X-ray Diffraction and Cryo-Electron Microscopy held at the Indian National Science Academy, New Delhi during15-17 December 2015.
2. Attended conference on IUBMB/RCB Advanced School on "Diabetes and Metabolic Syndrome: networks, crosstalks and interventions" held at the Heritage Village Resort, Manesar, Gurgaon, India, during 24-29 November 2014.
3. Attended conference on "3rd Summit of South Asian Academies & AASSA General Assembly" held at the Indian National Science Academy, New Delhi, India, during 14-17 October 2014.

Dr. Prasenjit Gucchait

1. Delivered seminar on "High-altitude hypoxia and immune responses" on THSTI World Immunology Day held on 29 April 2015.

Dr. Saikat Bhattacharjee

1. Attended International Symposium cum workshop titled Frontiers in Structural Biology: New Methods in X-ray Diffraction and Cryo-Electron Microscopy held at the Indian National Science Academy, New Delhi during15-17 December 2015.
2. Attended conference on IUBMB/RCB Advanced School on "Diabetes and Metabolic Syndrome: networks, crosstalks and interventions" held at the Heritage Village Resort, Manesar, Gurgaon, India, during 24-29 November 2014.
3. Attended conference on "3rd Summit of South Asian Academies & AASSA General Assembly" held at the Indian National Science Academy, New Delhi, India, during 14-17 October 2014.

Dr. Sam J. Mathew

1. Visited the Department of Human Genetics, University of Utah, USA, from 16 May-17 July 2015, as part of the "work outside the host institution" scheme of the Wellcome 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".
2. Attended conference and presented poster entitled "The role of Tcf4 and connective tissue fibroblasts in myogenesis" at the American Society for Cell Biology (ASCB) Meeting 2014, held at the Pennsylvania Convention Center, Philadelphia, Pennsylvania, USA, during 6-10 December 2014.
3. Delivered an invited lecture titled "A developmental perspective on metabolic syndrome" and participated in the IUBMB/RCB Advanced School on "Diabetes and Metabolic Syndrome: networks, crosstalks and interventions" held at the Heritage Village Resort, Manesar, Gurgaon, India, during 24-29 November 2014.

4. Attended conference and presented poster entitled "The role of developmental myosin heavy chain genes in skeletal muscle development, differentiation, regeneration and disease" at the Wellcome Trust/DBT India Alliance Fellows' Meeting 2014, organized at the Grand New Delhi, Vasant Kunj, New Delhi, during 4-5 November 2014.

Dr. Sivaram V. S. Mylavarapu

1. Delivered Distinguished Lecture titled "Motoring through Cell Division", at the India Innovation Research Centre, Delhi on 22 May 2015.
2. Delivered seminar titled "Regulation of Mitotic Fidelity by Molecular Motor Protein Subunits" atthe first South Asian Biotechnology Conference, Department of Biotechnology, South Asian University, New Delhi on 13 February 2015.
3. Delivered seminar titled "Regulation of Mitotic Fidelity by Molecular Motor Protein Subunits" at the 83rd Annual meeting of the Society of Biological chemists of India at Bhubaneshwar during18 -21 December 2014.
4. Attended International Symposium cum workshop titled Frontiers in Structural Biology: New Methods in X-ray Diffraction and Cryo-Electron Microscopy held at the Indian National Science Academy, New Delhi during15-17 December 2015.
5. Attended conference on IUBMB/RCB Advanced School on "Diabetes and Metabolic Syndrome: networks, crosstalks and interventions" held at the Heritage Village Resort, Manesar, Gurgaon, India, during 24-29 November 2014.

Dr. C. V. Srikanth

1. Attended as a co-organizer of the World Immunology Day celebration meeting held at THSTI, Faridabad on 29 April 2015.
2. Attended the Mini Indo-Canadian Immunology Symposium held at NII, New Delhi during 14-15 January 2015.
3. Presented a poster titled "Salmonella Engages host microRNAs for successful infection" at the India Alliance Fellows Meeting held at Hotel Grand in New Delhi during 5-6 November 2014.
4. Delivered a flash seminar and presented a poster titled "Salmonella wrestles with host by targeting SUMO: Complexity of life in the gut" at the International Conference organized by Indo-USA Science and technology Forum (IUSSTF) held at Irvine, California USA during 9-12 August 2014.

Dr. Vengadesan Krishnan

1. Delivered a talk on 'X-ray diffraction analysis of two crystal forms of backbone pilin SpaA fragment from Lactobacillus rhamnosus GG'in the National Symposium on Biophysics and Golden Jubilee Meeting of Indian Biophysical Society held at Jamia Millia Islamia, New Delhi during 14-17 February 2015.
2. Delivered an invited talk on 'Structural investigation of pilus proteins in Lactobacillus rhamnosus GG' in the South Asian Biotechnology Conference held at South Asian University, New Delhi during 12-14 February 2015.
3. Delivered an invited talk on 'Structural investigation of pili in beneficial bacteria' in the International Conference on Crystal Growth and Biomolecular crystallography held at SASTRA University, Tanjavur during 28-29 November 2014.

Memberships of Professional/ Academic Bodies/Editorial Boards

Dr. Dinakar M. Salunke

- Fellow, The World Academy of Sciences (TWAS)
- Fellow, Indian National Science Academy
- Vice President (International Affairs), Indian National Science Academy
- Fellow, National Academy of Sciences
- Fellow, Indian Academy of Sciences
- Member, Governing Council, National Brain Research Centre, Manesar
- Member, Governing Body, National Institute of Plant Genome Research, New Delhi
- Member, Governing Body, Translational Health Science & Technology Institute, Faridabad
- Independent Director, Biotechnology Industry Research Assistance Council (BIRAC) Board
- Member, Commission on Biological Macromolecules, International Union of Crystallography
- Member, Scientific Advisory Committee, National Brain Research Centre, Manesar
- Member, Scientific Advisory Committee for Biosciences and Bioengineering Group, IIT-Indore
- Chairman, INSA National Committee for International Union of Crystallography
- Member of Expert Committee for Life Sciences on Fund for Improvement of S&T Infrastructure in Higher Educational Institutions (FIST), Department of Science & Technology
- Member, Apex Committee, Biotechnology Industry Partnership Programme
- Member, Finance Committee, National Brain Research Centre, Manesar
- Member, Committee of Experts for Planning Synchrotron Radiation Sources, Office of the Principal Scientific Adviser, Government of India
- Chairman, Management Board, Technology Advancement Unit, DBT
- Member, Research Council, CSIR-IGIB, Delhi
- Member, Research Council, CSIR-CCMB, Hyderabad
- Chairman, Audit Committee, Biotechnology Industry Research Assistance Council
- Member, Indian Crystallographic Association

- Member, Indian Biophysical Society
- Member, Society of Biological Chemists
- Member, Association of Microbiologists of India

Dr. Sivaram V. S. Mylavarapu

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

Dr. C. V. Srikanth

- Member, American Association for Microbiology

Dr. Deepak T. Nair

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

Dr. Deepti Jain

- Member, Indian Crystallography Association
- Member, Society of Biological Chemists

Dr. Sam J. Mathew

- Member, American Society for Cell Biology (ASCB), for the year 2014

Dr. Saikat Bhattacharjee

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

Dr. Divya Chandran

- Member, American Society for Plant Biologists (ASPB)
- Review Editor, Frontiers in Plant Science: Plant Biotic Interactions, Frontiers Publishing Group

Dr. Vengadesan Krishnan

- Member, Indian Biophysical Society
- Member, Indian Crystallographic Association

Achievements & Academic Activities

Distinctions, Honours & Awards

1. Dr. Dinakar M. Salunke was elected fellow of the The World Academy of Sciences for the advancement of science in developing countries (TWAS).
2. Dr. C. V. Srikanth is member of the Infectious disease Biology Task Force of the DBT.
3. Dr. Tushar K. Maiti is member of the Human Development and Disease Biology Task Force of the DBT.
4. Dr. Deepak T. Nair was invited to give a mentor talk at the Annual Conclave of Ramalingaswami Fellows organized by Insitute of Life Sciences, Bhubaneshwar during 30 January-1 February 2015.
5. Dr. Sivaram Mylavarapu was invited to give a Distinguished Lecture at India Innovation research Centre, Delhi in May 2015.
6. Mr. Jithesh Kottur won the best poster award at the International Symposium cum workshop titled Frontiers in Structural Biology: New Methods in X-ray Diffraction and Cryo-Electron Microscopy held at the Indian National Science Academy, New Delhi during15-17 December 2015.
7. Mr. Pergu Rajaiah won the Gold Medal for Best Presentation at the 2nd AIST International Imaging workshop held at the Biomedical Research Institute, Tsukuba Science City, Japan in December 2014.
8. Ms. Shivlee Nirwal won the best poster award at the Indo-US conference meeting on Recent advances in Structural Biology and Drug Discovery (RASBDD-IIT-2014) at Indian Institute of Technology - Roorkee during 9-11 October 2014.
9. Mr. Jithesh Kottur, Ms Pranita Hanpude and Mr. Sagar Mahale won best poster awards at the RCB PAC meeting held during 30 September - 1 October 2014.

Achievements & Academic Activities

Publications

Original Peer Reviewed Articles

1. Bansal S, Singh M, Kidwai S, Bhargava P, Singh A, Sreekanth V, Kundu S, Singh R,andBajaj A (2014) Bile acid amphiphiles with tunable head groups as highly selective non-hemolytic antitubercular agents. ***Med Chem Commun*** 5: 1761.
2. Chandran D, Scanlon MJ, Ohtsu K, Timmermans MCP, Schnable PS and Wildermuth MC (2015) Laser microdissection-mediated isolation and in vitro transcriptional amplification of plant RNA. ***Curr Protoc Mol Biol*** 112:25A.3.1.
3. Chaurasia P, von Ossowski I, Palva A and Krishnan V (2015). Purification, crystallization and preliminary X-ray diffraction analysis of SpaD, a backbone-pilin subunit encoded by the fimbrial spaFED operon in Lactobacillus rhamnosus GG. ***Acta Cryst*** F71: 103.
4. Da Q, Teruya M, Guchhait P, Teruya J, Olson JS, Cruz MA (2015) Free hemoglobin increases von Willebrand factor-mediated platelet adhesion in vitro: implications on circulatory devices. ***Blood*** (in press).
5. Hallstrom KN, Srikanth CV, Agbor TA, Dumont CM, Peters KN, Paraoan L, Casanova JE, Boll EJ, McCormick BA (2015) PERP, a host tetraspanning membrane protein, is required for Salmonella-induced inflammation. ***Cell Microbiol*** 17:843.
6. Jain A and Salunke DM (2015) Purification, identification and preliminary crystallographic studies of an allergenic protein from Solanum melongena. ***Acta Crystallogr*** F71:221.
7. Keefe AC, Lawson JA, Flygare SD, Fox ZD, Colasanto MP, Mathew SJ, Yandell M and Kardon G (2015) Muscle stem cells contribute to myofibres in sedentary adult mice. ***Nature Commun*** 14:7087.
8. Kottur J, Sharma A, Gore KR, Narayanan N, Samanta B, Pradeepkumar PI and Nair DT (2015) Unique Structural Features in DNA Polymerase IV enable efficient bypass of the N^2-Adduct induced by the Nitrofurazone antibiotic. ***Structure*** 23:56.
9. Kumar S, Bhargava P, Sreekanth V and Bajaj A (2015) Design, synthesis, and physico-chemical interactions of bile acid derived dimeric phospholipid amphiphiles with model membranes. ***J Colloid Interface Sci*** 448:398.
10. Sharma A, Pohane A, Bansal S, Bajaj A, Jain V and Srivastava A (2015) Cell penetrating synthetic antimicrobial peptides (SAMPs) exhibiting potent and selective killing of mycobacterium by targeting its DNA. ***Chem Eur*** J21:3540.
11. Singh K, Verma V, Yadav K, Sreekanth V, Kumar D, Bajaj A and Kumar V (2015) Design, regioselective synthesis and cytotoxic evaluation of 2-aminoimidazole-

quinoline hybrids against cancer and primary endothelial cell. ***Eur J Med Chem*** 87: 150.

12. Singh M, Bansal S, Kundu S, Bhargava P, Singh A, Motiani RK, Shyam R, Sreekanth V, Sengupta S and Bajaj A (2015) Synthesis, structure-activity relationship, and mechanistic investigation of lithocholic acid amphiphiles for colon cancer therapy. ***Med Chem Commun*** 6: 192.
13. Singh M, Kundu S, Reddy A, Sreekanth V, Motiani RK, Sengupta S, Srivastava A and Bajaj A (2014) Injectable small molecule hydrogel for localized and sustained in vivo delivery of Doxorubicin. ***Nanoscale*** 6:12849.
14. Singhal R, Annarapu GK, Pandey A, Chawla S, Ojha A, Gupta A, Cruz MA, Seth T and Guchhait P (2015) Hemoglobin interaction with GP1ba induces platelet activation and apoptosis: a novel mechanism associated with intravascular hemolysis. ***Haematologica*** (in press).
15. Verma S, Mohapatra G, Ahmed SM, Rana S, Jain S, Khalsa JK and Srikanth CV (2015) Salmonella engages host microRNAs to modulate SUMOylation: a new arsenal for intracellular survival. ***Mol Cell Biol*** 35:2932.
16. Weinert T, Olieric V, Waltersperger S, Panepucci E, Chen L, Zhang H, Zhou D, Rose J, Ebihara A, Kuramitsu S, Li D, Howe N, Schnapp G, Pautsch A, Bargsten K, Prota AE, Surana P, Kottur J, Nair DT, Basilico F, Cecatiello V, Pasqualato S, Boland A, Weichenrieder O, Wang BC, Steinmetz MO, Caffrey M and Wang M (2015) Fast native-SAD phasing for routine macromolecular structure determination. ***Nature Methods***12:131.
17. Yadav K, Bhargava P, Bansal S, Singh M, Sreekanth V and Bajaj A (2015) Nature of the charged head group dictates the anticancer potential of lithocholic acid-tamoxifen conjugates for breast cancer therapy. ***Med Chem Commun*** 6:778.

Reviews

1. Agarwal M, Kumar P and Mathew SJ (2015) The Groucho/Transducin-like enhancer of split protein family in animal development. ***IUBMB Life*** 67:472.
2. Bhattacharjee S, Noor JJ, Gohain B, Gulabani H, Dnyaneshwar IK, and Singla A. (2015) Post-translational modifications in regulation of pathogen surveillance and signaling in plants: The inside - (and perturbations from) outside story. ***IUBMB Life*** 67: 524.
3. Chandran D (2015) Co-option of developmentally regulated plant SWEET transporters for pathogen nutrition and abiotic stress tolerance. ***IUBMB Life*** 67:461.
4. Hanpude P, Bhattacharya S, Dey AK and Maiti TK (2015) Deubiquitinating enzymes in cellular signaling and disease regulation. ***IUBMB Life*** 67:544.
5. Jain D (2015) Allosteric control of transcription in GntR family of transcription regulators: A structural overview. ***IUBMB Life*** 67:556.
6. Kaur H and Salunke DM (2015) Antibody promiscuity: Understanding the paradigm shift in antigen recognition. ***IUBMB Life*** 67:498.

7. Krishnan V (2015). Pilins in gram-positive bacteria: A structural perspective. ***IUBMB Life*** 67:533.

8. Kumar M, Pushpa K and Mylavarapu SVS (2015). Splitting the Cell, Building the Organism: Mechanisms of Cell Division in Metazoan Embryos. ***IUBMB Life*** 67: 575.

9. Kundu S, Kumar S and Bajaj A (2015) Cross-talk between bile acids and gastrointestinal tract for progression and development of cancer and its therapeutic implications. ***IUBMB Life*** 67:514.

10. Nair DT, Kottur J and Sharma R (2015) A rescue act: Translesion DNA synthesis past N2-deoxyguanosine adducts. ***IUBMB Life*** 67:564.

11. Pandey A, Chawla S and Guchhait P (2015) Type-2 Diabetes: Current Understanding and Future Perspectives. ***IUBMB Life*** 67: 506.

12. Verma S, Srikanth CV (2015) Understanding the complexities of Salmonella-host crosstalk as revealed by *in vivo* model organisms. ***IUBMB Life*** 67:482.

Achievements & Academic Activities

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 Colloquium lecture was delivered by Prof. G. B. Nair (Executive Director, THSTI). Prof. Nair is an eminent microbiologist who has made seminal contributions regarding the pathophysiology and virulence associated genetics of enteric pathogens. The title of his talk was "Genomes and Public Health- The Cholera Example." The lecture was attended by scientists of all ages from different institutions located in NCR.

Achievements & Academic Activities

Distinguished Lecture Series

With the objective of creating an international platform for discussions and debates in different research areas of Biology and Biotechnology, eminent scientists from national and international institutions are invited to deliver lectures in RCB. These lectures are usually attended by young, mid-career and senior scientists. In addition, post-graduate and undergraduate students and teachers are also invited to these talks. The speakers are generally scientists who have achieved great success in their fields and they share thoughts, ideas and experimental strategies for their field of research and science in general.

In the past year, the following eminent scientists have delivered lectures:

1. Prof. John E. Johnson (The Scripps Institute, San Diego, USA) "Studies of Assembly and Maturation of dsDNA Viruses reveal novel mechanisms driving biological dynamics" on 9 October 2014.
2. Prof. Mauro Giacca (Director-General, ICGEB, Trieste, Italy), "New Genes for Old Hearts" on 10 December 2014.
3. Prof. Ronald D. Vale (University of California, San Francisco & Howard Hughes Medical Institute), "Biology's Nanoscale Motors" on 16 March 2015.

Achievements & Academic Activities

Faculty Mentorship Programme

The faculty at RCB are being mentored by senior scientists in their respective domain areas of research. The mentors are distinguished scientists who have made important contributions in their area of research. The mentors spend one day in RCB interacting with their mentees research group and provide detailed feedback regarding the ongoing research at both specific and general levels. A formal report is prepared and sent to the mentee and the PAC. The programme has been operational for two years now and the faculty and the PAC members agree that it has been an unqualified success. The Centre is grateful to all mentors for taking time out from their busy schedules to help the scientific programs of RCB.

Joint UNESCO-DBT Symposium

A symposium on "Role of Biotechnology in post-2015 Development Agenda" organised jointly by UNESCO and RCB was held on 13 February 2015 in Paris.

The symposium addressed new developments in biotechnology, which will be critical in the endeavour of the UN to achieve the organizations millennium development goals. A panel comprising of Ms. Irina Bokova, (Director General, UNESCO), Ms. Ruchira Kamboj (Ambassador & Permanent Representative of India to UNESCO), Prof. K.VijayRaghavan (Secretary, Department of Biotechnology, Govt. of India), Dr. Dinakar M. Salunke (Executive Director, Regional Centre for Biotechnology), Dr. Satyajit Rath (Senior Scientist, National Institute of Immunology) and Dr. Mauro Giacca (Director General, International Center for Genetic Engineering and Bioechnology) discussed the importance of Biotechnology in sustainable development for all.

During the symposium, the Director General highlighted the role of the Regional Centre for Biotechnology- a category 2 Centre of UNESCO- in fostering innovation and training of manpower in the area of Biotechnology. In addition, the Centre will enable synergistic interactions between government, civil society, scientists and the common man to develop policy and agenda for effective and safe application of developments in biotechnology. Prof. K. VijayRaghavan outlined India's strategy to enhance the role of biotechnology to meet challenges related to health, agriculture and environment. He also emphasised the government's commitment towards development of novel technologies that will be accessible to all.

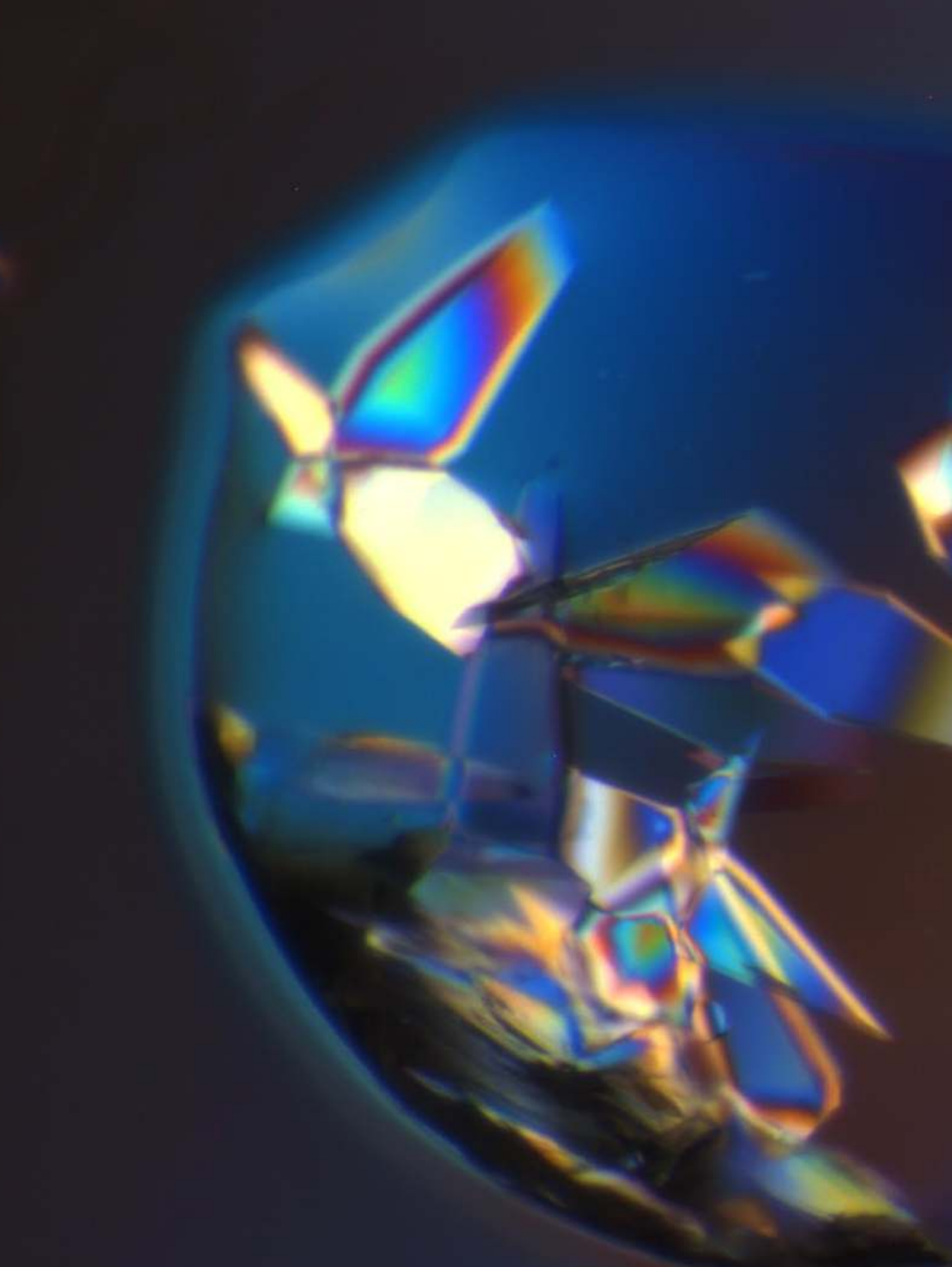
Dr. Satyajit Rath described the new experiment being implemented in India of developing goal based programmes in biotechnology in a cluster model. Dr. Dinakar Salunke provided a description of the core goals of RCB and an overview of the ongoing activities at the Centre. In addition, he shared the role that RCB is destined to play in shaping all aspects of Biotechnology Research and Innovation in the near and distant future. Dr. Mauro Giacca elaborated on the international efforts of the ICGEB in dissemination of new developments in biotechnology for the benefit of humankind.

Seminars delivered by Visiting Scientists

S.No.	Name of Scientists & Affiliation	Seminar Title	Date
1.	Dr. Bela Desai The Scripps Research Institute, USA	Sixth Sense: Understanding proprioception using the fly model	08/09/2015
2.	Dr. Vijay C. Verma Texas A & M University, USA	Tailoring the endosphere microbiome of plants for enhanced stress resistance and performance	28/08/2015
3.	Dr. Himanshu Sinha Tata Institute of Fundamental Research, Mumbai	Defining Genotype to Phenotype Relationships: The Next Steps	27/08/2015
4.	Dr. Shri Ram Yadav Indian Institute of Technology-Roorkee	A near death experience during phloem cell differentiation and symplastic cell-cell communication during Arabidopsis vascular development	20/08/2015
5.	Dr. Tanmay Majumdar Georgia Regents University, USA	Microbiota based nutritional therapy: A new arsenal against *Helicobacter pylori* induced obesity	10/08/2015
6.	Dr. Lipi Thukral Institute of Genomics and Integrative Biology, New Delhi	Exploring Biomolecular Recognition using Multiscale Computer Simulations	23/04/2015
7.	Dr. Hemchandra Jha University of Pennsylvania, USA	Role of Gammaherpesviruses in cancer progression	09/04/2015
8.	Prof. Ronald D. Vale University of California San Francisco, USA	Biology's Nanoscale Motors	16/03/2015
9.	Prof. Aneel K. Aggarwal Chemical and Structural Biology, Mount Sinai School of Medicine, USA	DNA polymerases and Cancer: An Inside View	26/12/2014
10.	Prof. Mauro Giacca International Center for Genetic Engineering & Biotechnology, Italy	New genes for old hearts	10/12/2014
11.	Dr. Santosh Chauhan University of New Mexico, USA	Molecular mechanisms and regulation of autophagy	08/12/2014
12.	Dr. Sachin Kumar Cincinnati Childrens Research Foundations, USA	The Role of GTPases in neutrophil migration during inflammation	04/12/2014
13.	Dr. Ashok Sharma University of Nebraska Medical Center, USA	Epigenetic Activation and Oncogenic Function of POTE Genes in Human Epithelial Ovarian Cancer (EOC)	02/12/2014
14.	Dr. Praveen K. Vermula Institute of Stem Cell Biology and Regenerative Medicine, Bangalore	Disease-responsive biomaterials to prevent inflammatory diseases	20/11/2014

S.No.	Name of Scientists & Affiliation	Seminar Title	Date
15.	Dr. Subhas Samanta University of Pittsburgh, USA	Light active small molecules to control biomolecular structures and functions	20/11/2014
16.	Dr. Nagaraj Balasubramanian, IISER, Pune	Adhesion Dependent Regulation of Caveolar Trafficking: Role in Mechanosensing and Cancers	07/11/2014
17.	Prof. Narayana V. L. Sthanam University of Alabama, Birmingham, USA	Complement evasion by bacteria for survival	05/11/2014
18.	Dr. Dimple Notani University of California San Diego, USA	Role of Transcriptional Enhancers and their Hierarchical Networks in Gene Regulation	29/10/2014
19.	Dr. Arnab Ray National Cancer Institute, USA	Coping with Stress: How Replication forks contend with Genotoxic Insult-Implications for Cancer chemoresistance	09/10/2014
20.	Prof. John E. Johnson The Scripps Institute, USA	Studies of Assembly and Maturation of dsDNA Viruses reveal novel mechanisms driving biological dynamics	07/10/2014

Achievements & Academic Activities



Extramural Activities & Funding

Extramural Activities & Funding

Multi-institutional Preterm Birth (PTB) Programme

A national level innovative multi-institutional study is implemented through RCB, which is an important partner on the pre-term 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 vaginal microbial landscape. The program involves development and evaluation of putative biomarkers, identification of simple microbiological tool based vaginal risk factors, modulation of vaginal microbiota for therapeutic purpose and evaluation of environmental modifications chosen from SNP analysis. Some of the major public health concerns addressed by the program are biological risks associated with foetal growth and PTB, and clinical consequences of PTB and intra uterine growth retardation.

PTB program actively involves bridging expertise from diverse fields, such as, paediatrics, gynaecology, infectious disease biology, epidemiology, microbiology, immunology, platform technologies, cellular & molecular biology, genetics, statistics and computational & systems biology. A cross-disciplinary approach will be used to elucidate possible mechanisms and outline the etiology of PTB. Whole-genome screens, and temporal genomics, epigenomics and proteomics studies will be performed to assess the biological risk factors and dynamic nature of PTB. Vaginal microbial flora will be profiled using a metagenomics approach and information generated 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 for 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 team also comprises of a clinical team and a project management team. The clinical team will be stationed at Gurgaon General Hospital, which is the site of study, and comprises of the clinical coordinator, research physicians, nurses, attendants, field workers and field supervisors.

RCB's primary role is to address the proteomics based questions. Proteomic analysis will identify the differentially regulated proteins and protein function alterations due to posttranslational modifications in the preterm birth condition. The clinical team has been collecting samples since May 2015 and patient recruitment is occuring on regular basis. The mass spectrometry system has been upgraded for high throughput application and initial few control samples have been tested for quality control and standardization. The ultimate goal of RCB's approach is to decipher the molecular mechanisms contributing to the poor pregnancy outcome, based on proteomics studies.

Extramural Activities & Funding

NCR Biotech Science Cluster, Faridabad

RCB is an important component in the NCR Biotech Science Cluster (NCR-BSC) set up in Faridabad. The Biotech Science Cluster 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 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 into 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 biosafety containment laboratories.

Advanced Technology Platform Centre (ATPC)

RCB is playing a key role in the setting up of a state-of-the-art Advanced Technology Platform Centre (ATPC). 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 Biotech Science Cluster in Faridabad. The ATPC is an initiative for multidisciplinary research that will translate scientific and technological advancements into innovations for public health improvement.

Biotechnology Incubator

The biotechnology Incubator is being established as part of the Biotech Science Cluster in partnership with BIRAC. This state of the art facility would provide new and emerging companies with a compatible environment to 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 provides 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 a common office.

International Networking

AIST, Japan

The Regional Centre for Biotechnology (RCB) and the National Institute of Advanced Industrial Science & Technology (AIST), Japan entered into a partnership for capacity building initiatives in bio-imaging and biotechnology in the year 2014. This initiative enhances 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.

The Memorandum of Agreement will enable RCB and AIST to jointly organize advanced research training in bio-imaging and biotechnology, including *in vivo* and *in vitro*

imaging, cryo-electron microscopy, crystallography and similar technologies. Key features of the agreement are: to set up a DBT-AIST International Laboratory for Advanced Bio-imaging (DAILAB) at RCB,to facilitate joint collaborations engaging Indian and Japanese scientists and, to support selected Indian researchers for training in specialized areas of bio-imaging and biotechnology at AIST as well as other laboratories outside AIST in Japan.

The agreement provides opportunities to both institutions to develop research collaborations and promote capacity building training, which will benefit young scientists not only from India and Japan but also from the UNESCO member countries in the Asia-Pacific and SAARC regions. Indeed, through the current initiatives, RCB is poised for broadening its horizons and will facilitate bridging science and knowledge dissemination for betterment of mankind.

Centre for Molecular Medicine of the Karolinska Insitutet, Sweden

RCB is collaborating with the Centre for Molecular Medicine (CMM) of the Karolinska Insitutet, Sweden for an innovative Post Doctoral programme and exchange programme on Biomedical and Molecular medicine.

DBT will sponsor a program where Indian post-docs will be selected and sent to CMM for training in various ongoing research projects. The Post-doc program shall be for a minimum period of 1 year and a maximum of 2 years. The assistance for the Post-doc will cover the stipend & travel (to & fro for individual alone) at the time of joining & leaving the Institute. A joint symposium will also be held by the partner institutions in India and Sweden to show case the expertise and share knowledge in the areas.

University of Toronto, Canada

Regional Centre for Biotechnology along with partner institutions in the Biotech Science Cluster has initiated collaboration with the Department of Immunology, University of Toronto in the area of immunology and human health. The aim of such a partnership is to offer basic education and training to scientific personnel within participating institutes using traditional and modern methods. The education and training initiatives will develop innovative courses that impart skills required for cutting-edge-research in the fields of immune cell signalling, host-pathogen interactions, patient sampling and bio-banking and comparison of neonatal and adult immune systems. When it comes to force, the partnership will enable short term visits for PhD students, post-doctoral researchers and faculty members between the institutes to enable cross-fertilization of ideas and efforts to fuel new discoveries in the area of Immunology.

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 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 number of publications in leading international journals. In late 2014, the project was extended for a further two years and

Extramural Activities & Funding

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.

International and National Responsibilities

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 conducive environment for the planning and implementation of R&D projects focussed on product development and technology transfer. RCB manages the TAU and as apart of its multi-dimensional role as an inter-institutional coordinator, has hosted the TAU and provided administrative facilities for its functioning.

Biosafety Support Unit

The Department of Biotechnology, Ministry of Science and Technology, has entrusted RCB with the responsibility of establishing a Biosafety Support Unit (BSU), which will develop guidelines and protocols for generating biosafety data to address the 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. The unit would provide appropriate scientific information on emerging issues and, in addition, a periodical on Biosafety Regulation would also be published for knowledge dissemination in this important area.

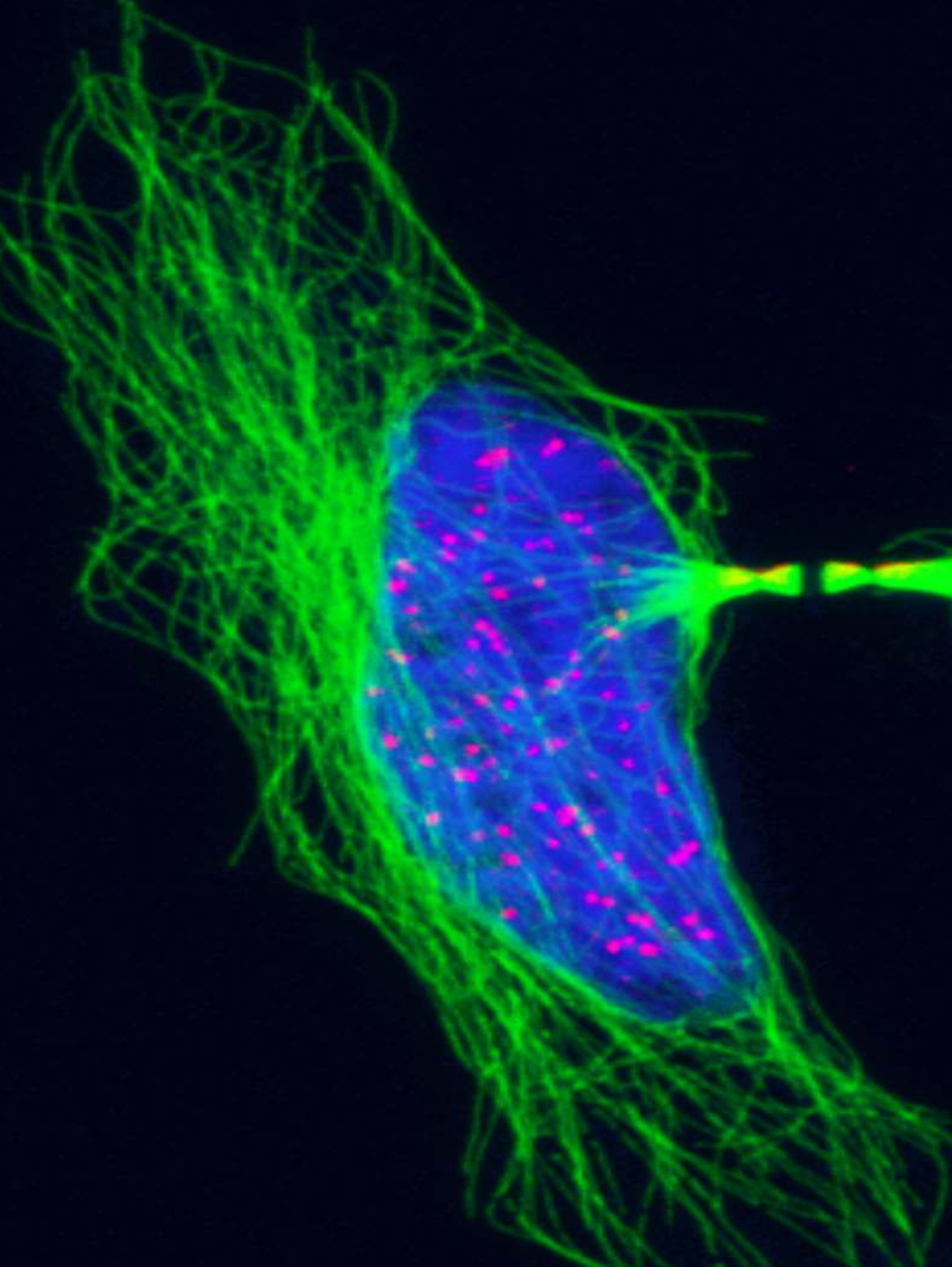
Extramural Activities & Funding

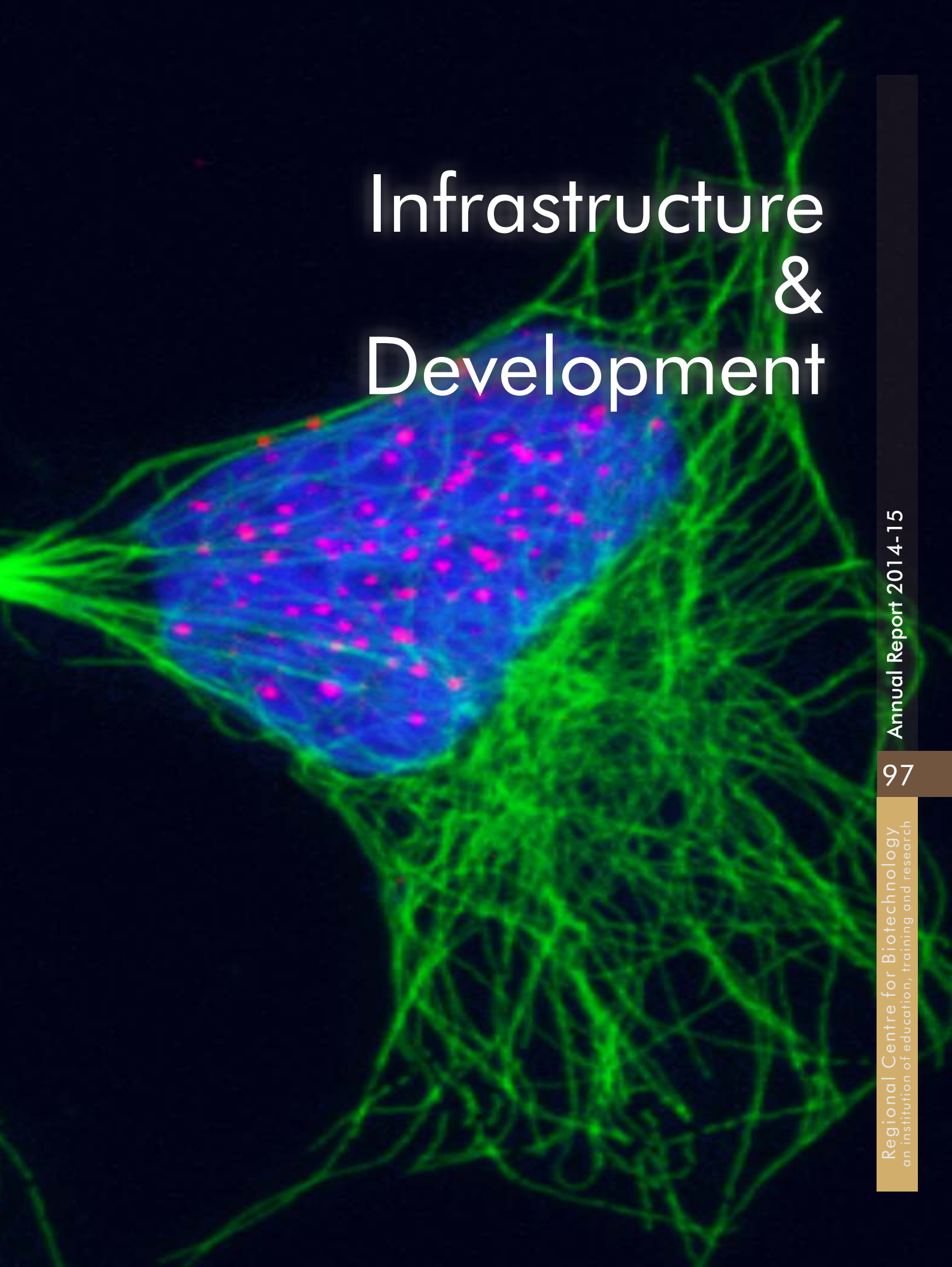
Extramural Grants

1. Dr. C. V. Srikanth, "Understanding Salmonella-mediated alterations in host SUMOylation: implications in infection and inflammation", Rs. 3.28 Cr (Wellcome Trust/DBT India Alliance Intermediate Fellow)
2. Dr. Sam J. Mathew, "Role of developmental myosin heavy chains in skeletal muscle development, regeneration, homeostasis and disease", Rs. 3.50 Cr (Wellcome Trust/DBT India Alliance Intermediate Fellow)
3. Dr. Puspha Kumari, "Understanding role of Exocyst complex in cell division and development in *Caenorhabditis elegans*", Rs. 1.44 Cr (Welcome Trust/DBT India Alliance Early Career Fellow)
4. Dr. Avinash Bajaj, "Engineering of Nanomaterials and their interactions with DNA and Cell Surface", Rs. 73 lakhs (Ramanujan Fellow, DST)
5. Dr. Saikat Bhattacharjee, "Elucidating inositol-dependent signalling routes of effector-triggered immunity for identifying new approaches for engineering crop resistance against diverse pathogens", Rs. 82 lakhs (Ramalingaswami Fellow, DBT)
6. Dr. Deepti Jain, "Structure and mechanism of FleQ, master regulation of transcription of flagellar and biofilm genes in *Pseudomonas aeruginosa*" Rs. 52 lakhs (Innovative Young Biotechnologist Awardee, DBT)
7. Dr. Dinakar M. 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, NII, Safdarjung Hospital & General Hospital, Gurgaon Rs. 48.85 Cr (DBT)
8. Dr. Avinash Bajaj, "Engineering of Nanomaterials for Combination Cancer Therapy", Rs. 30 lakhs (DBT)
9. 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", Rs.79.05 lakhs (DBT)
10. Dr. Prasenjit Guchhait, "Pathophysiology of thrombocytopenia in dengue infection", jointly with AIIMS, New Delhi, Rs. 55 lakhs (DBT)
11. Dr. Sivaram Mylavarapu, "Molecular Basis for Silencing of the Spindle Assembly Checkpoint", Rs. 35 lakhs (DBT)
12. Dr. Avinash Bajaj, "Phospholipid based Nanomaterials as Novel Therapeutics for Cancer", Rs. 29 lakhs (DBT)
13. Dr K. Vengadesan, "Structural investigations of surface nano scale assembly in a gut bacterium", Rs. 60 lakhs (DBT)
14. Dr. Sam J. Mathew, "The role of MET-CBL signaling in Rhabdomyosarcoma", Rs 24.54 lakhs (DBT).
15. Dr. Tushar K. Maiti, "Targeting ubiquitin proteasome system for the anticancer drug development: A peptoid based inhibitor design, synthesis and evaluation", Rs 24.54 lakhs (DBT)

16. Dr. Deepak T. Nair, "Effect of N2-adducts of deoxyguanosine of DNA synthesis by replicative and translesion DNA polymerases", Rs. 57 lakhs jointly with IIT-Bombay (DBT)
17. Dr. Dinakar M. Salunke, "Establishment of NCR Biotech Science Cluster (NCR-BSC)" jointly with THSTI, NII, NIPGR and NBRC, Rs. 47.06 Cr (DBT)
18. 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" jointly with NII & JNCASR, Rs. 5.0 Cr (DBT)
19. 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, Rs. 1.83 Cr (DBT)
20. 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, Rs. 1.62 Cr (DBT-BMFG-BIRAC-USAID Award of Grand Challenge India-All Children Thriving)

Extramural Activities & Funding





Infrastructure & Development

Laboratory Infrastructure

RCB is equipped with modern infrastructure to conduct research and training in different areas of biology and biotechnology. The facilities available at RCB include:

Microscopy and Imaging: This 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 a 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: 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) in RCB 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 are present in RCB. 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.

Each spacious laboratory in RCB is shared between two PIs and has laboratory benches, storage furniture, seating space, PI cabins along with internet and phone access. All laboratories have equipment required to prepare samples that is based on the respective PIs area of research.

The Centre has rooms reserved for laboratory meetings, interactions, discussions and teaching. One of the seminar rooms in the auditorium complex of the NCR-BSC is dedicated to RCB academic activities.

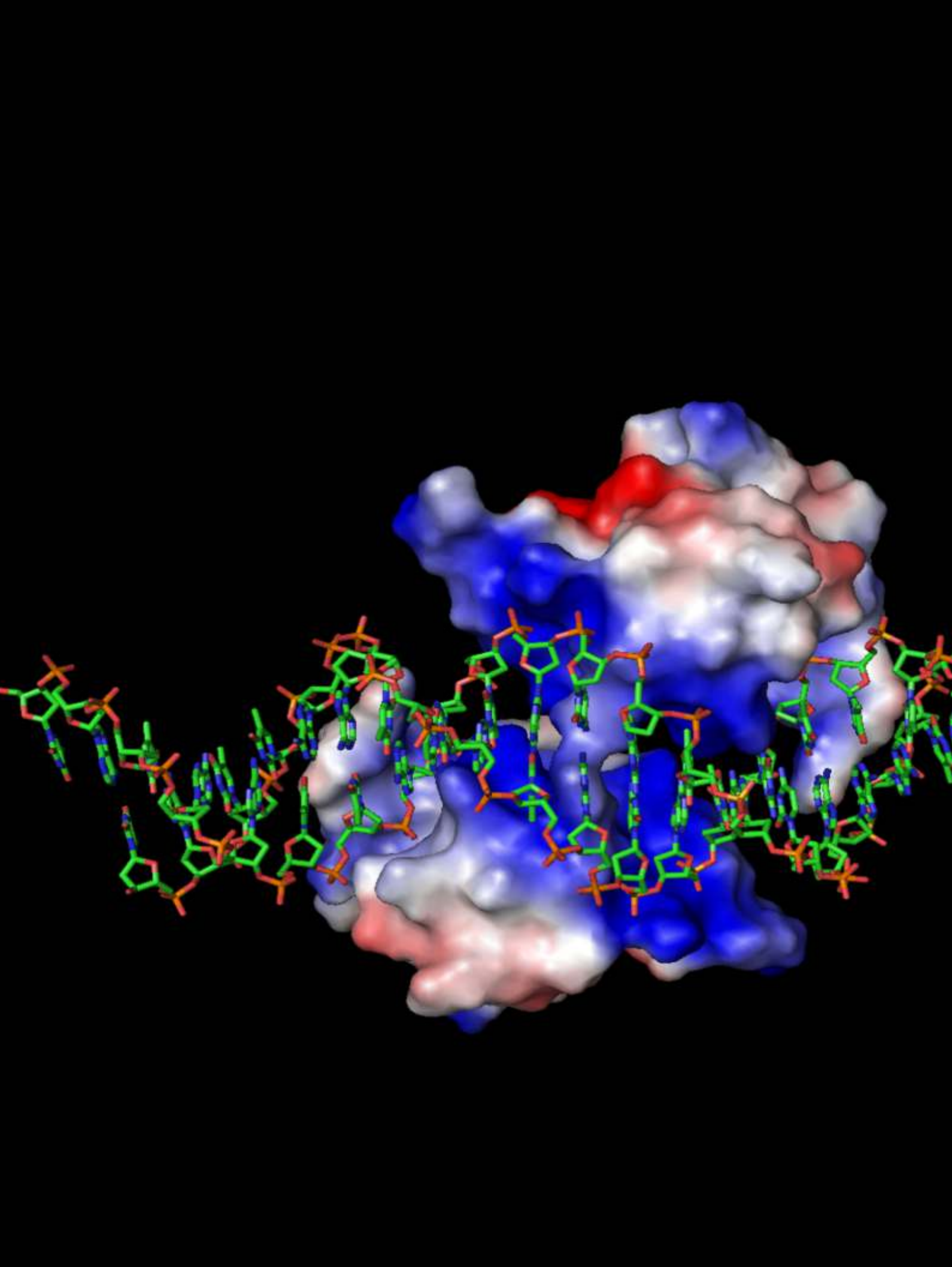
Infrastructure & Development

Information Technology infrastructure

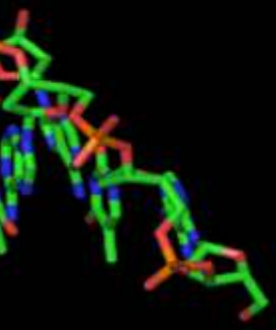
During the year 2014-15, after shifting to the new campus, the requirement to establish a state-of-art IT network and infrastructure was of paramount importance due to the isolated location of the NCR-BSC. The task was undertaken in right earnest and has been completed successfully. LAN and Wi-fi connectivity has been provided to all users and the high speed internet connectivity through the National Knowledge Network has also been successfully established, with active cooperation & guidance of the Officers of the NIC cell in DBT. Library & e-library facility has been fully established at RCB with regular subscription of print and electronic versions of scientific journals. The access to a wide range of e-journals provided by the DBT Electronic Library Consortium (DELCON) is also available in the library. The centre is also equipped for video-conferencing and we aim to enhance this facility in the near future.

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 Governments initiative of the "Digital India Campaign". A very competent & experienced IT service support team has been put in place and the Centre is also in the process of developing & implementing a highly attractive, user-friendly and dynamic web-site.

Infrastructure & Development



Financial Statements



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

AUDITOR'S REPORT

We have audited the attached Balance Sheet of REGIONAL CENTER FOR BIOTECHNOLOGY, 3rdMilestone, Faridabad Gurgaon Expressway, Faridabad as on**31st March, 2015**, 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 standards generally accepted in India. Those standards requirethat 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 to the explanation give to us, the said accounts give a true and fair view:

   a) In case of the Balance Sheet, of the Statement of Affairs of the center as at 31.03.2015 and

   b) In case of Income & Expenditure account, of the center during the period ended on 31st March 2015

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: 29/09/2015

Srivastava Kumar & Co. NEW DELHI Chartered Accountants

REGIONAL CENTRE FOR BIOTECHNOLOGY

3rd Milestone, Faridabad Gurgaon Expressway, Faridabad

BALANCE SHEET AS AT 31ST MARCH, 2015

Amount (In Rs.)

CORPUS / CAPITAL FUND AND LIABILITIES	Schedule		Current Year		Previous Year
CORPUS / CAPITAL FUND	1	-	198,315,046.90	-	204,677,052.00
RESERVES AND SURPLUS	2	-	415,012.00	-	415,012.00
CURRENT LIABILITIES AND PROVISIONS	3	-	122,654,713.00	-	126,467,880.00
BIOTECH SCIENCE CLUSTER (BSC)	3	-	1,799,442,768.00	-	1,564,446,410.00
TOTAL			**2,120,827,539.90**		**1,896,006,354.00**
ASSETS				-	
FIXED ASSETS	4	-	128,236,911.90	-	127,318,900.00
FUNDS IN SHORT TERM DEPOSITS	5	-	24,825,000.00	-	24,825,000.00
CURRENT ASSETS,LOANS ADVANCES ETC.	5	-	347,696,638.00	-	309,333,231.00
BIOTECH SCIENCE CLUSTER (BSC)	5		1,620,068,990.00		1,434,529,223.00
a. Capital Work in progress		1,314,766,254.00		1,095,540,338.00	
b. Advance to BSC construction.		295,793,698.00		330,752,035.00	
c. Funds in short term deposits		3,400,000.00		3,400,000.00	
d. Accrued interest & TDS		6,109,038.00		4,836,850.00	
TOTAL			**2,120,827,539.90**		**1,896,006,354.00**
SIGNIFICANT ACCOUNTING POLICIES					
CONT. LIABILITIES & NOTES ON ACCOUNTS					

AS PER OUR SEPRATE REPORT
OF EVEN DATE ATTACHED
SRIVASTAVA KUMAR & CO
CHARTERED ACCOUNTANTS

Sd/-
BIJU MATHEW
SENIOR MANAGER (A&F)

Sd/-
DINAKAR M SALUNKE
EXECUTIVE DITECTOR

Sd/-
RASHMI GUPTA
PARTNER

PLACE: Gurgaon
DATE: 29/09/2015

Financial Statements

REGIONAL CENTRE FOR BIOTECHNOLOGY

3rd Milestone, Faridabad Gurgaon Expressway, Faridabad

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31st MARCH, 2015

Amount (in Rs.)

Income	**Schedule**	**Current Year**	**Previous Year**
Grants/Subsides	6	134,806,692.00	113,969,218.00
Fees/Subcrpitions	7	227,720.00	268,094.00
Interest on investments on fixed deposits	7	4,805,118.00	2,315,830.00
Deferred Income-Fixed Assets		66,362,005.10	68,609,870.00
TOTAL (A)		**206,201,535.10**	**185,163,012.00**
EXPENDITURE			
Establishment Expenses	8	48,971,484.00	34,786,959.00
Other Administrative Expenses etc.	9	91,860,107.00	81,766,183.00
Excess of Expenditure Carried over		(992,061.00)	
Depreciation (Net Total at the year end-corresponding to schedule 4)		66,362,005.10	68,609,870.00
TOTAL(B)		**206,201,535.10**	**185,163,012.00**
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
SRIVASTAVA KUMAR & CO
CHARTERED ACCOUNTANTS

Sd/-
BIJU MATHEW
SENIOR MANAGER (A&F)

Sd/-
DINAKAR M SALUNKE
EXECUTIVE DITECTOR

Sd/-
RASHMI GUPTA
PARTNER

PLACE: Gurgaon
DATE: 29/09/2015

Financial Statements

Regional Centre for Biotechnology

Accounting Policies and Notes Forming Parts of the Balance Sheet and Income & Expenditure Account for the Year Ended on 31st March, 2015

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. 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. (a) Recurring Grants have been recognised in the Income & Expenditure account and non-recurring Grants have been shown as part of capital.
   (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. 663,62,005.00.
4. (a) The depreciation has been provided w.e.f. the date of purchase 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.
5. 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.
6. 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.
7. 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.
8. 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)
9. 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. 19,61,962.00. as overheads to different projects.

10. Against the Grants-in-aid General amounting to Rs. 725.00 lakhs received for incurring recurring expenditure during the year, Rs. 208.08 lakhs provision against purchase orders outstanding for 2013-14, interest income generated by the Centre for an amount of Rs. 48.05 lakhs and Rs.2.28 lakhs as miscellaneous income, the centre has booked an expenditure of Rs.993.27 lakhs during the year and has further liability of Rs. 29.14 lakhs by way of outstanding purchase orders.
11. No provision has been made by the Institute towards the gratuity payable and other terminal benefits to staff.
12. The balances of the previous year have rearranged as per requirement and shown in Balance Sheet against the relevant heads.
13. The Institute has received Rs. 172,42,87,768.00 (including RCB) from various institutes for the construction of campus at Faridabad and the total expenditure incurred as on 31st March 2015 against such contribution is amounted to Rs. 161,05,59,952.00 (Rs.131,47,66,254.00 being the booked as Capital Work-in-progress and & Rs. 29,57,93,698.00 being advanced to the Project Monitoring Consultant).

(Rs. In lakhs)

Sl. No	Constituent Partner	Opening Balance as on 1.4.2014	Received during 2014-15	Total receipts on 31.3.2015
1.	THSTI	7209.30	674.00	7883.30
2.	NII	1491.00	388.02	1879.02
3.	RCB	5100.65	400.00	5500.65
4.	Bio-Incubator	1304.00	0.00	1304.00
5.	ATPC	50.00	0.00	50.00
6.	Interest on investment of BSC funds	489.51	136.39	625.90
	Total	**15644.46**	**1598.41**	**17242.87**

14. The Capital Work-in-progress booked in the accounts includes the already constructed laboratory buildings of THSTI, RCB & NII and the under construction buildings of ATPC, Bio-incubator, the hostel & 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. Dinakar. M. Salunke)
Executive Director

Rashmi Gupta
Partner

Place: Gurgaon
Date: 29/09/2015


RCB

Institutional Information

Board of Governors

1.	Prof. K. VijayRaghavan Secretary Department of Biotechnology Ministry of Science & Technology, Govt. of India New Delhi	Chairperson
2.	Mr. Shigeru Aoyagi Director & UNESCO Representative to Bhutan, India, Maldives and Sri Lanka UNESCO Office New Delhi	Member
3.	Prof. Akhilesh K. Tyagi Director National Institute of Plant Genome Research New Delhi	Member
4.	Dr. Dinakar M. Salunke Executive Director Regional Centre for Biotechnology NCR Biotech Science Cluster Faridabad	Convener
5.	Mr. S. Sinha Advisor Department of Biotechnology Ministry of Science & Technology, Govt. of India New Delhi	Special Invitee

Programme Advisory Committee

1.	Prof. Angelo Azzi Senior Scientist Tufts University 711 Washington St. Boston MA 02111, USA	Chairperson
2.	Prof. Subrata Sinha Director National Brain Research Centre NH8, Nainwal Mode Manesar - 122051	Member
3.	Dr. Satyajit Rath Senior Scientist National Institute of Immunology Aruna Asaf Ali Marg, JNU Campus New Dellhi-110067	Member
4.	Prof. K. Veluthambi Senior Professor School of Biotechnology Madurai Kamaraj University Palkalai Nagar Madurai-625021	Member
5.	Shri S. Sinha Advisor Department of Biotechnology MoS&T, Govt. of India 6th – 8th Floor, Block-2, CGO Complex Lodhi Road, New Delhi-110003	Member
6.	Dr. K. V. S. Rao Group Leader International Centre for Genetic Engineering and Biotechnology ICGEB Campus, Aruna Asaf Ali Marg New Delhi-110067	Member

7.	Dr. G. B. Nair Executive Director Translational Health Science & Technology Institute NCR Biotech Science Cluster Faridabad	Member
8.	Prof. T. P. Singh Distinguished Biotechnology Professor Department of Biophysics All India Institute of Medical Sciences (AIIMS) Ansari Nagar, New Delhi-110029	Member
9.	Prof. Joel Sussman Senior Professor Department of Structural Biology The Weizmann Institute of Science Rehovot, Israel 76100	Member
10.	Prof. Keichi Namba Professor & Head Protonic NanoMachine Group Graduate School of Frontier Biosciences Osaka University, 1-3 Yamadaoka, Suita, Osaka-565-0871 Japan	Member
11.	Prof. R. Venkata Rao Vice Chancellor National Law School of India University Teachers Colony, Chandra Layout Bangalore-560242, Karnataka	Member
12.	Dr. Dinakar M. Salunke Executive Director Regional Centre for Biotechnology NCR Biotech Science Cluster Faridabad	Member Secretary
13.	Dr. Saman Habib Senior Principal Scientist Central Drug Research Institute Lucknow	Special Invitee
14.	Dr. Deepak Gaur Associate Professor School of Biotechnology Jawaharlal Nehru University New Delhi	Special Invitee

Executive Committee

1.	Dr. Dinakar M. Salunke Executive Director Regional Centre for Biotechnology NCR Biotech Science Cluster Faridabad	Chairperson
2.	Mr. Shigeru Aoyagi Director & UNESCO Representative to Bhutan, India, Maldives and Sri Lanka UNESCO Office New Delhi	Member
3.	Mr. Rakesh Ranjan Joint Secretary Ministry of Human Resource Development Govt. of India New Delhi	Member
4.	Mr. S. Sinha Advisor Department of Biotechnology Ministry of Science & Technology, Govt. of India New Delhi	Member
5.	Mr. Tanmaya Lal Joint Secretary (UNES) Ministry of External Affairs Govt. of India New Delhi	Member

Finance Sub-Committee

1.	Dr. Dinakar M. Salunke Executive Director Regional Centre for Biotechnology NCR Biotech Science Cluster Faridabad	Chairperson
2.	Dr. G. B. Nair Executive Director Translational Health Science & Technology Institute NCR Biotech Science Cluster Faridabad	Member
3.	Mr. J. B. Mohapatra Joint Secretary & Financial Advisor Department of Biotechnology Ministry of Science & Technology, Govt. of India New Delhi	Member
4.	Mr. S. Sinha Advisor Department of Biotechnology Ministry of Science & Technology, Govt. of India New Delhi	Member
5.	Dr. Satyajit Rath Senior Scientist National Institute of Immunology Aruna Asaf Ali Marg, JNU Campus, New Delhi	Member

Scientific Personnel

Faculty

Executive Director

Dr. Dinakar M. Salunke

Associate Professors

Dr. Prasenjit Guchhait
Dr. Deepak T. Nair
Dr. Avinash Bajaj

Assistant Professors

Dr. Deepti Jain
Dr. Divya Chandran
Dr. Saikat Bhattacharjee
Dr. Sam J. Mathew
Dr. Sivaram V. S. Mylavarapu
Dr. Chittur V. Srikanth
Dr. Tushar Kanti Maiti
Dr. Vengadesan Krishnan

Emeritus Scientists

Prof. S. V. Eswaran

International Adjunct Faculty

Prof. Falguni Sen

Young Investigators

Dr. Amit Kumar Yadav
Dr. Bornali Gohain
Dr. Masum Saini
Dr. Megha Kumar
Dr. Rashi Gupta
Dr. Sheetal Chawla
Dr. Smriti Verma
Dr. Suneel Kumar Tripathi
Dr. Vaibhav Kumar Pandya

Wellcome DBT 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. Sarita Chandan Sharma
Mr. Salman Ahmad Mustfa
Ms. Priyanka Chaurasia
Ms. Rashi Singhal
Ms. Kavita Yadav
Mr. Manhar Singh Rawat
Mr . Rahul Sharma
Ms. Shivlee Nirwal
Mr. Jithesh Kottur
Mr. Naveen Narayanan
Ms. Mary K Johnson
Mr. Deepankar Singh
Ms. Shilpi Nagpal

Junior Research Fellows

Ms. Chanchal
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. Sanjay Pal
Ms. Sunayana Dagar
Ms. Abhin Kumar Megta
Ms. Akashi

Ms. Sulagna Bhattacharya
Ms. Meha Shikhi
Mr. Priyajit Banerjee
Mr. Syed Mohd. Aamir Suhail
Ms. Rajnesh Kumari Yadav
Mr. Ingole Kishore Dnyaneshwar
Mr. Megha Gupta
Mr. Pharvendra Kumar
Ms. Minakshi Sharma
Ms. Raniki Kumari
Ms. Hridya Chandrasekar
Ms. Swarnima Pandey
Ms. Shreyasi Das
Ms. Manisha Kumari
Ms. Arunima Gupta
Mr. Krishnendu Goswami
Ms. Shachi Saluja
Mr. Mritunjay Kasera
Mr. Zaid Kamal Madni
Mr. Chandan Kumar
Mr. Anuj

Research Associates/ Post Doctoral Fellows

Dr. Mukesh Kumar
Dr. Amit Kumar
Dr. Jewel Jameeta Noor
Dr. Deepak Kumar Jangir
Dr. Mritika Sen Gupta
Dr. Amit Kumar Dey
Dr. Arjun Kumar Misra
Dr. Ashish Kumar
Dr. Shivendra Pratap
Dr. Bhoj Kumar

Project Assistants

Ms. Navneet Kaur
Mr. Avinash Gupta
Ms. Anuska Das
Mr. Pavit Kumar
Mr. Abhishek Kumar Singh
Ms. Neha Sharma
Ms. Komalla Varsha
Ms. Dipika Nandi
Mr. Sharad Vashist
Mr. Bahadur Singh Gurjar
Mr. Ankur Kumar
Ms. Shraddha K. Dahale

Scientific Officer – Projects

Mr. Suneel Prajapati

Institute Management

Academic Management

Executive Director
Dr. Dinakar M. Salunke

Registrar
Dr. B. Chandrasekar

Documentation Assistants
Mr. Deepak Kumar
Ms. Vaishali Mangla

Administration & Finance

Executive Director
Dr. Dinakar M. Salunke

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. Mehfooz 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

Consultants

Science & Technology

Dr. Ramesh Juyal
Dr. Ujjaini Dasgupta
Dr. Nirpendra Singh

Finance

Mr. C. L. Raina

Information Technology

Ms. Alka Chug

Engineering

Mr. Shyam Sunder Budhwar

Junior Consultants

Mr. Jagdish Chander
Mr. Ashok Kumar Singhal

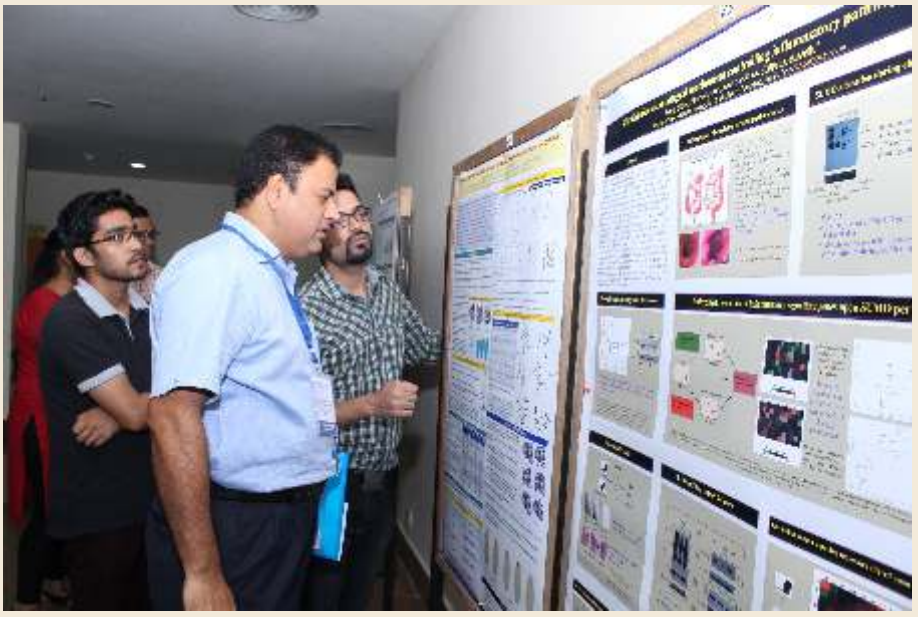
Institutional Information

Memories

Independence Day celebrations

Memories

Poster session during PAC meeting



Memories


The Exocyst complex is required for germline development and embryogenesis in Caenorhabditis elegans

Tree Plantation By Prof. K. VijayRaghavan (Secretary, DBT)

Memories