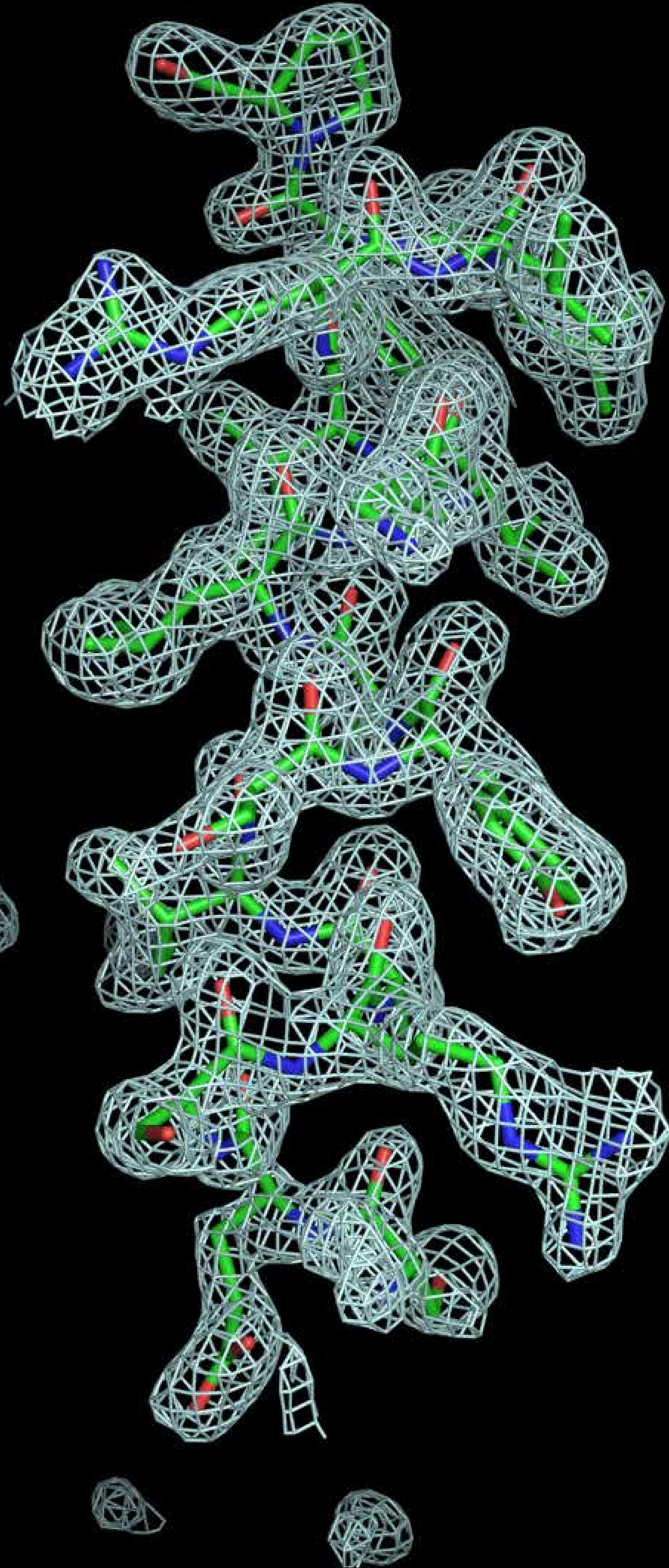
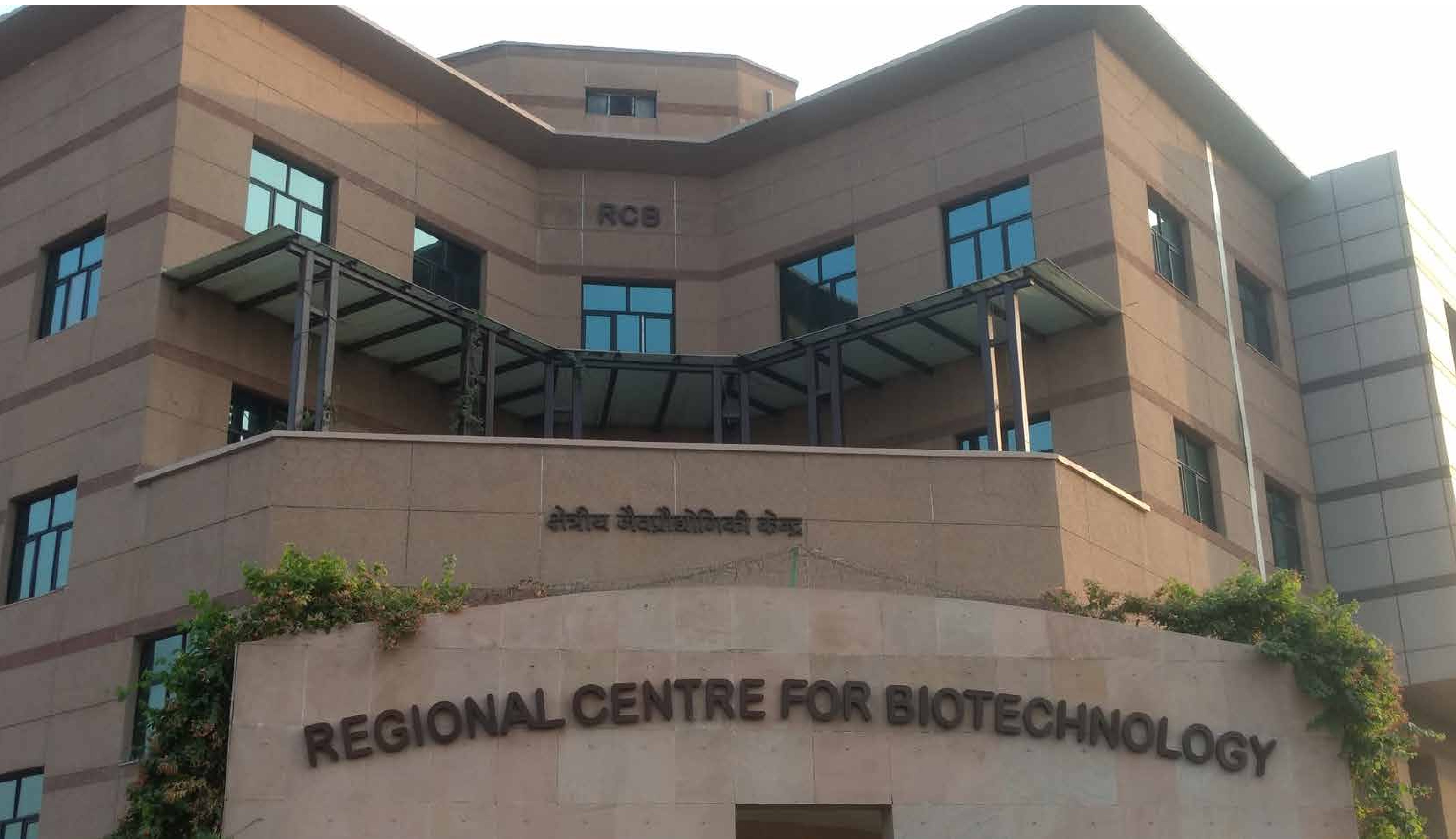
United Nations
Educational, Scientific and
Cultural Organization

क्षेत्रीय जैव प्रौद्योगिकी केन्द्र
Regional Centre
for Biotechnology



Annual Report
2016-2017


RCB
REGIONAL CENTRE FOR BIOTECHNOLOGY

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From the Executive Director's Desk

Regional Centre for Biotechnology (RCB) was conferred the status of an institution of national importance by the Indian Parliament in July 2016, and over the last one year the Centre has worked tirelessly to frame the RCB statutes, ordinances and regulations. Having been approved by the respective authorities, these have now been published in the Gazette of India and are being presented to the Indian Parliament. This would complete the process of giving the formal structure to RCB as an academic institution with the authority to grant degrees in various specialities of biotech sciences. Accordingly, RCB launched its very own doctoral programme this year where over 20 students were admitted to work towards their PhD degree. Admission to an integrated MSc-PhD programme in Biotechnology has also been announced; the programme will start from July 2018.

RCB continues to provide opportunities for young scientists by offering innovative education and training programmes that contribute to the human resource development in the advanced areas of life sciences and biotech sciences. An International Symposium-cum-Workshop on "Structural proteomics of Macromolecular Complexes using X-ray crystallography and Mass spectrometry" was organized at RCB in December 2016 that brought together eminent scientists, researchers from the finest institutions from around the globe to exchange ideas. RCB organized a joint mini-symposium with Advanced Institute of Science and Technology (AIST) of Japan on "Cellular Mechanisms in Health and Disease" in February 2017. Jointly with THSTI, RCB hosted the 5th Molecular Virology meeting in February 2017. The meeting brought together researchers from various Indian institutions working in the various areas of virology.

RCB continues to be a category-2 institution of the UNESCO; the linkage providing an international reach to our academic and training programmes. RCB is working with Merck, in partnership with African Union, Ethiopian Ministry of Health and UNESCO, to train researchers from the African continent. A student each from Kenya and Cameroon were trained at RCB under this programme this year. We are also working with UNESCO to organize the review of the Asian Biotechnology School and exploring possibilities for participation of RCB in this programme. Under the auspices of the Department of Biotechnology (DBT) of Government of India, and the European Synchrotron Radiation Facility (ESRF) Council, RCB signed a three-year arrangement for the medium-term scientific use of synchrotron radiation for non-proprietary research, with focus in structural biology, to provide access to the ESRF to Indian scientists. This programme has provided tremendous support to the Indian structural biologists and has benefitted a large number of young research students.

The Centre has continued to make advances in the various research areas being pursued by its principal investigators. Besides, RCB continues to participate in a multi-institutional research programme aimed to understand the biology of pre-term birth to identify possible biomarkers to predict the outcomes. A large cohort of pregnant women has been established by THSTI at the Gurgaon Civil Hospital and the scientists at RCB are conducting a comprehensive study on the proteome of the various tissue samples from these women.

The various scientific programmes of RCB can be broadly grouped under the following heads: Infectious Disease Biology, Molecular Medicine, Cancer Biology and Therapeutics, and Agricultural Biotechnology. The scientific reports section of the annual report provides details of progress made under the various programmes. Notable progress in a couple of areas is highlighted below.

Bacterial motility is mediated by the action of flagella that are complex dynamic structures composed of numerous proteins. The assembly of functional flagella requires coordinated expression of about 40 genes for synthesis of its structural and regulatory components and the associated chemosensory apparatus. FleN, a P loop ATPase is vital for maintaining a monotrichous phenotype in Pseudomonas aeruginosa. FleN exhibits

antagonistic activity against FleQ, the master transcriptional regulator of flagellar genes. Crystal structures of FleN revealed that it undergoes drastic conformational changes on ATP binding to attain a structure capable of dimerization. The study revealed that ATP-induced structural remodeling facilitated the formation of the functional dimer in FleN and helped the antiactivator attain a reversible form that could calibrate FleQ activity to an optimal level.

Current membrane-targeting antimicrobials fail to target Mycobacteria due to their hydrophobic membrane structure, ability to form drug-resistant biofilms, and their natural intracellular habitat within the confines of macrophages. Synthetic antimicrobial polymers (SAMPs) derived from biocompatible polyamides were engineered that could target drug-sensitive and drug-resistant Mycobacteria with high selectivity. This has implications for developing novel therapies to treat Mycobacterial infections.

Finally, I would like to acknowledge the full cooperation from my colleagues in the RCB faculty and administration, and complete support from DBT and UNESCO, the members of the Board of Governors, the Programme Advisory Committee and the various other statutory committees in accomplishing the various scientific and academic activities at the Centre, and I look forward to their continued support in the future.

Sudhanshu Vrati
Executive Director

Mandate of the Regional Centre for Biotechnology

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

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

The objectives of the Regional Centre are:

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

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

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

d. to create a hub of biotechnology expertise and to address human resource needs in the countries in the region,

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

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

The functions of the Regional Centre are:

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

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

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

d. to undertake research and development and scientific investigations in collaboration with relevant research centres in the region,

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

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

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

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

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

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

Scientific
Reports

Molecular Determinants of Genomic Integrity and Plasticity

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The genome houses the blueprint of life and any changes in this blueprint in the form of mutations are generally harmful. Conversely, beneficial variations caused primarily by mutations arise in living organisms under conditions of stress. The aim of the group is to understand how the integrity of the genome is maintained and also unearth the underlying cause of variations in the blueprint. The ability of pathogens to evolve under conditions of stress is responsible for the onset of multi-drug resistance and failure of vaccines. These studies will therefore enable development of new strategies to combat antimicrobial resistance and pandemic virus infections, two major public health problems that plague the world currently.

Molecules that either maintain genetic integrity or render genomic plasticity are investigated in this group. The efforts will provide mechanistic insight into how organisms evolve and adapt to the environment. For all cellular processes to function optimally, the integrity of the genome has to be maintained. Conversely, plasticity in the genome can relieve selection pressures 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 latter group has been implicated in the onset of drug resistance and immune evasion in pathogens. The antagonistic action of these two different sets of molecules ensure that genomic plasticity is calibrated to endow adaptive capability without severely compromising genetic viability. We aim to elucidate the structural mechanism utilized by different molecular determinants of genomic integrity and plasticity to achieve function.

With this broad aim, the following biological processes are under scrutiny in this program: (a) DNA replication & translesion DNA synthesis (b) Stress-induced mutagenesis (c) DNA mismatch repair (d) Stress-induced epigenetic modification (e) Transposition and (f) Replication of the genome of Japanese Encephalitis and Chikungunya viruses. The studies will provide insight into how variation appears in the genotype and phenotype of organisms, especially in response to stress. The insight gained from these studies will also provide a robust platform for the development of novel therapeutic strategies against pathogenic bacteria and viruses.

Chemical Mechanism of high fidelity DNA synthesis

In all living organisms, deoxyribonucleic acid (DNA) is synthesized by DNA polymerases and these enzymes catalyze template-directed synthesis of DNA. DNA polymerases employ semiconservative mode of replication using primer-template duplex DNA and deoxynucleotide triphosphates (dNTPs) as precursors for DNA synthesis. The primer provides a 3′-hydroxyl group that can be extended by the polymerase and the identity of the incoming dNTP is determined by the template residues. Mg^{2+} ions also play an important role in the polymerization reaction. DNA polymerases extends the primer in the 5′-3′ direction. The formation of a phosphodiester bond between the -phosphate of the incoming dNTP and the 3′- hydroxyl group of the terminal primer nucleotide is the primary chemical reaction catalyzed by the DNA polymerase enzyme.

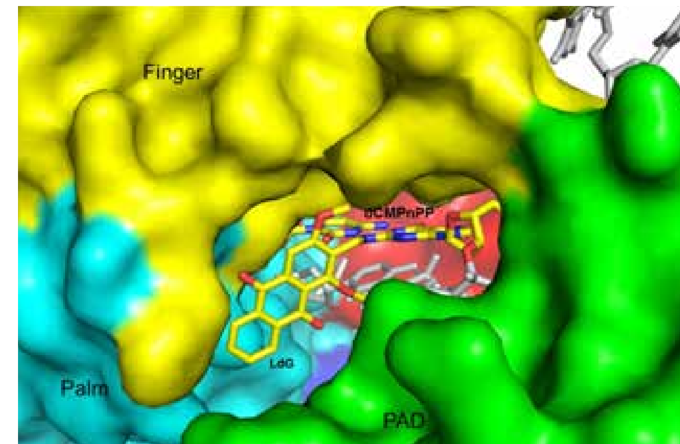

Figure 1: Surface representation of $PolIV_{LdG:dCMPnPP}$ structure. The surface of PolIV is displayed with the palm, fingers and PAD region colored cyan, yellow and green, respectively. The DNA and dCMPnPP are displayed in stick representation. Lucidin moiety occupies the channel formed between fingers domain and PAD region.

Time-resolved crystallography was conducted on DNA polymerase IV from Escherichia coli to elucidate the steps involved in the formation of a phosphodiester bond during DNA synthesis by a DNA polymerase. The study will track the birth of a phosphodiester bond in the DNA polymerase active site.

Translesion bypass of N^2-adducts

Lucidin is a hydroxyanthraquinone metabolite that is found naturally in plants such as the Madder root. This compound is a known mutagen and can react with the N^2- atom of deoxyguanosine to form a bulky minor groove adduct. The lucidin-N^2-deoxyguanosine adduct is known to destabilize DNA and affect genomic processes such as replication. Here the study showed that DNA polymerase IV from Escherichia coli can bypass this bulky N^2-adduct accurately with high efficiency. The enzyme is able to add the correct incoming nucleotide dCTP opposite the adduct and extend past the DNA lesion to rescue replication stalled at this adduct. The structure of PolIV in complex with DNA bearing the lesion at the templating position and dCMPnPP ($PolIV_{LdG:dCMPnPP}$) shows the presence of a channel formed between the fingers, PAD and palm domains of the enzyme that can be occupied by bulky minor groove adducts such as LdG (fig. 1). The LdG adduct is present at an angle with respect to the DNA helical axis to ensure that there are no steric clashes formed with the enzyme atoms. The structure and allied biochemical assays with wild type and mutant protein also show that the residues S42 and F76 play an important role in stabilizing the adduct in the active site in a conformation compatible with productive catalysis. These residues are unique to PolIV and bacterial and archaeal orthologs that lack these residues are unable to bypass the LdG lesion. Overall, PolIV possesses unique structural features that allow accurate and efficient bypass of bulky N^2-adducts such as LdG. Crystals of DNA polymerase IV with DNA bearing N2-dG adducts of varying sizes have been obtained and structure determination and analysis is currently in progress.

DNA Mismatch Repair

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

This study shows that in the absence of DNA and in the presence of adenosine diphosphate (ADP) or adenylyl imididiphosphate (AMPPNP), MutS forms a symmetric dimer wherein a large gap exists between the monomers through which DNA can enter the central channel. The mismatch scanning monomer (B_m) then moves by nearly 50 Å to associate with the other monomer (A_m) and due to this movement the N-terminal domains of both monomers press onto DNA to bend it.

The mechanism of toroid formation evinces that the force required to bend DNA arises primarily due to the movement of the monomer B_m and hence, the MutS dimer acts like a pair of pliers to bend DNA (fig. 2). The study also sheds light on the allosteric mechanism that influences the expulsion of ATP from A_m on DNA binding. Overall, this study provides mechanistic insight regarding the primary event in DNA mismatch repair i.e. the assembly of the MutS-DNA complex.

Stress-induced epigenetic modification

There is an abundance of restriction modification (RM) systems in the gastric pathogen Helicobacter pylori.

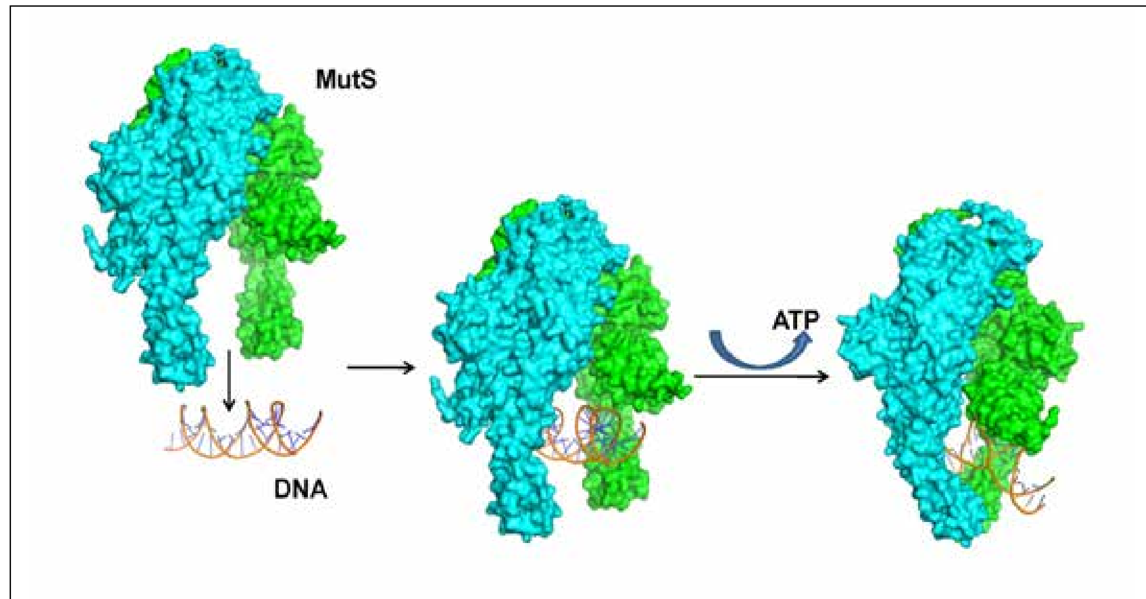

Figure 2: Mechanism of assembly of the MutS-DNA complex. The different stages in the assembly of MutS-DNA complex are displayed. The two monomers are shown in surface representation, labelled A_m and B_m and are coloured cyan and green, respectively.

These RM systems regulate natural transformation and consequent genomic plasticity of this microbe. Some DNA Methyltransferases (dMtases) that are part of these RM systems exhibit activity only in adverse conditions. Methylation of cognate sequences by these enzymes results in the alteration of transcriptional profiles to rapidly respond to stress in the environment. The aim in this study is to elucidate the regulatory mechanisms that permit these enzymes to act only under specific environmental conditions.

The expression of the HP0593 dMtase is upregulated when the pathogen encounters low pH. HP0593 is a 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. The crystal structure of HP0593 in complex with the inhibitor sinefungin (SFG) was determined (fig. 3). The structure along with biochemical and biophysical analysis of site-specific mutants of HP0593 suggests that the functional form of this enzyme is a tetramer that 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. In addition to HP0593, other stress-induced dMtases have been purified and crystallization trials are in progress.

Figure 3: Structure of HP0593:sinefungin complex. The structure of the monomer of HP0593 in complex with inhibitor sinefungin is displayed.

Methods in Macromolecular Crystallography

The group is also involved in improving methods of structure determination and analysis. Previously, the group had participated in a study to develop a rapid protocol for structure determination using the weak anomalous signal from sulphur atoms in proteins. Recently the overall quality of structures deposited in the PDB was assessed and this exercise suggests that the application of extremely rigid criteria to assess structures would lead to unnecessary rejection, at the initial stages itself, of potentially useful data that may otherwise give rise to important discoveries.

Biology of Infectious and Idiopathic Inflammation of the Gut

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Inflammation involves a very complex cascade of molecular events, understanding of which may be crucial for betterment of clinical diagnosis and therapy. A food-borne bacterial pathogen, Salmonella Typhimurium that causes gastroenteritis in humans is under study to discern key molecular pathways of host-pathogen crosstalk. Using state-of-the-art tools of microbiology, molecular biology and fluorescent imaging, the study identified a novel molecular-pathway involved in inflammation in two very varied forms of gut inflammation. These pathways are now being probed for mechanistic details. The ultimate goal is not only to combat drug-resistant infections but also to find therapeutic solutions to auto-immune disorders.

This research program is focused on understanding the molecular mechanisms that shape infection, inflammation and autoimmune disorders of the gut using a model intracellular gastric pathogen, Salmonella Typhimurium. Here using a multipronged approach involving the cell culture model, mouse model and human patient samples, novel molecular mechanisms that govern inflammation in various forms of gut illnesses are being investigated. As a part of this program, inflammatory diseases of the gut that arise due to Salmonella infection, and immune dysfunction such as Crohn's disease and Ulcerative colitis, are being examined. Specifically, the significance and exact role of SUMOylation in Intestinal Bowel Disease (IBD) and Salmonella disease is being studied. In addition, use of components of the SUMOylation machinery, or other pathways that are linked to SUMOylation, as possible targets of therapeutic interventions against gut inflammation are also to be explored.

SUMOylation perturbation severely affects intracellular life of Salmonella

Salmonella Typhimurium (ST) is a gram-negative bacterium that infects humans and animals via the oral route by the consumption of contaminated food or water. The disease caused by ST in humans, termed gastroenteritis, involves diarrhoea, abdominal cramps and fever. Such food-borne illnesses pose a major health challenge for developing nations like India. A recent report arising from this program revealed that ST infection led to a down modulation of host cell SUMOylation, a post-translational modification pathway. Further this work revealed that SUMOylation perturbation resulted in a compromised infection and altered inflammatory pathways. SUMOylation is highly conserved and is central to the regulation for various cellular processes. Three SUMO paralogues (SUMO1, SUMO2 and SUMO3) are present in mammals. The addition of SUMO to the target substrate requires sequential enzymatic action of E1-enzyme (SAE1/SAE2 heterodimer), E2-enzyme (UbC9) and one of several E3-ligases that act on specific targets. SUMOylation has been shown to participate in several fundamental cellular processes including replication, transcription and genome maintenance but has not been fully explored in gut illnesses. Attempts towards understanding the connection between SUMOyaltion, Salmonella infection and intestinal inflammation are reported herewith.

The effect of experimental SUMO-perturbation at longer durations post Salmonella infection was

investigated. Experimental up-regulation of any of the SUMO machinery components led to serious effects on the intracellular multiplication of bacteria as revealed by colony forming units (c.f.u.) assay. Since intracellular life of Salmonella depends on members of vesicular transport pathway proteins (VTPP), VTPP expression was probed during Salmonella infection in the context of the SUMOylation machinery. In SUMOylation machinery perturbed epithelial cells, as anticipated, the levels of global SUMOylation were much higher than those of the corresponding control cells. Among VTPP, Rab7 levels were negatively affected in SUMOylation perturbed infected cells. To understand the underlying mechanisms, the cellular protein synthesis machinery was blocked by using the inhibitor cycloheximide. These experiments revealed that Rab7 stability was adversely affected (upto 25 %) when the cellular SUMOylation machinery was perturbed. To understand the details of SUMOyaltion-dependent effect on Rab7 stability, a proteasome inhibitor MG132 was added to infected and SUMOylation-perturbed infected cells. Interestingly, treatment of cells with MG132 prevented the Rab7 turnover process in all conditions, thus linking Rab7 degradation to the proteasomal pathway. Proteins destined to undergo proteasomal degradation are known to be ubiquitylated. Accordingly, in the case of Rab7, a subtle increase in ubiquitin-modification was observed in infected samples. Moreover, a much more pronounced Rab7 ubiquitination was observed in SUMOylation-perturbed infected samples, which explained its lowered stability in SUMO-perturbed conditions. Together these experiments revealed that SUMO-perturbation results in a reduced stabilty of Rab7 and its degradation occurs via a ubiquitination-dependent proteasomal pathway.

To identify the proteins that undergo alteration in their SUMOylation during ST infection a comparative tandem mass-spectrometry (MS/MS) based assay system was developed. The method involved enrichment of SUMOylated proteins by an affinity purification system followed by MS/MS analysis. The obtained affinity-purified samples were concentrated, de-salted, processed and MS/MS assays performed using an AB SciEx 5600 mass spectrometer. Based on this comparative proteomics approach, the ST infection-specific SUMO-proteome was identified. A significant number of proteins that displayed altered SUMOylation in Salmonella infected samples compared to controls were observed. The analysis revealed various interesting candidates including several transcription factors, regulators of cell signalling and endocytic pathway components. These proteins are now being examined in detailed to understand the significance of SUMO-alteration.

SUMOylation in intestinal autoimmune diseases

Crohn's disease (CD) and Ulcerative Colitis (UC) are diseases that involve aberrant immune activation resulting in a major healthcare challenge. A cell culture model, a mouse model and human CD and UC patient samples were used to study the role of SUMOylation. Briefly, dextran-sulphate-sodium induced colitis in mice (DSS-mice) displayed intestinal inflammation and alteration of global SUMOylation with significant lowering of the E2-SUMO enzyme, Ubc9. DSS-mice with severely downregulated Ubc9 displayed exacerbated inflammation, thus linking lowered SUMO-status with inflammation. Comparative proteome analysis of healthy and inflamed mouse intestines revealed a distinct SUMOylome in DSS-mice with critical alteration of SUMOylated forms of key cellular regulators, including Akt1. Lowering of Ubc9 in murine primary-epithelial culture led to lowered Akt1 activity and pronounced proinflammatory signalling. A dramatic lowering of colonic SUMO-status was also seen in human CD and UC patient biopsies. Patients with maximum disease indices displayed severely lowered SUMOylation status and reduced levels of SUMOylated-Akt1. Thus in the absence of Akt1 SUMOylation, a lowered or inefficient wound healing in epithelial tissue was observed. Together these results point towards a novel mechanism underlying IBD-pathophysiology through Akt1-SUMOylation crosstalk.

These studies have revealed a crucial role for a pathway called SUMOylation, an inherent cellular protein modification that can alter the fate of the target. Experiments involving a mouse model of colitis and human IBD patient samples demonstrated the existence of aberrant SUMOylation pathways leading to a global molecular signaling. These findings, the first of their kind in case of IBD, connect two very important cellular mechanisms (SUMOylation and Akt1) to intestinal inflammation and highlight them as potential targets for therapeutic intervention.

A crucial role for SUMOylation in two major intestinal illnesses of humans has been established. In the future, the immediate goal is to understand the altered SUMO-proteome and its significance in gut inflammation. The role of deSUMOylases, the enzymes responsible for removing the SUMO-conjugation of proteins, in these diseases will also be investigated. Changes in the gut microbiome in IBD and colorectal cancer will also be explored. Possibilities of using SUMOylation machinery components, such as Ubc9 and Pias1, as a means for therapeutic intervention for treatment of inflammatory diseases of the gut will be finally tested.

Transcription Regulation: Structure and Mechanism

Dr. Deepti Jain

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The ability to perceive a stimulus and respond appropriately is the hallmark of any living organism. Bacteria respond to environmental stimuli by translocating to a more favourable milieu. Bacterial motility is mediated by the action of flagella that are complex dynamic structures composed of numerous proteins. Flagella are molecular motors that are whip-like structures present on the bacterial cell surface and are important not only for imparting movement but also play a vital role in bacterial virulence, biofilm formation and host colonization. This research group investigates atomic level details of the various regulators that influence flagellar assembly in pathogenic bacteria like Pseudomonas aeruginosa. Understanding the regulatory pathway of flagellar genes is essential, as it will provide a robust platform to develop novel therapeutic strategies against nosocomial pathogens such as Psedomonas aeruginosa.

Transcription is a fundamental process of life that endows an organism with the ability to respond to external stimuli and internal cues by modulating its gene expression profile. In bacteria, the process of transcription is tightly regulated via modulators. These factors may be global or specific depending on the number of genes and range of cellular functions that they modulate. Research in this group focuses on structural studies of macromolecular complexes involved in bacterial gene expression. The major objectives of research in this programme include studying the transcription regulation of the flagellar gene network in Pseudomonas aeruginosa, understanding the mechanism utilized by single subunit RNA polymerase to overcome oxidative stress in mitochondria and comprehending the mechanism of stress-induced crosstalk between two component systems involved in antibiotic resistance in Staphylococcus aureus.

Mechanism utilized by single subunit RNA polymerase to overcome the oxidative stress in mitochondria

Single subunit RNA polymerases (RNAPs) 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. Mitochondrial transcription in Arabidopsis thaliana is mediated by two single-subunit (~100 KDa) RNA polymerases – RpoTm and RpoTmp. RpoTm (AtRPOTm) 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. Mitochondria are the major sites for the generation of reactive oxygen species, which can cause damage to the proteins, lipids, carbohydrates and nucleic acids in the mitochondria. The oxidative environment of mitochondria can hydrolyze the most abundant rNTP species of ATP and GTP to 8-oxo-GTP and 2-hydroxy-ATP respectively, thus leading to oxidized RNA molecules in the cell. Moreover, the mitochondrial DNA (mtDNA) is not protected by nucleosomes (as opposed to nuclear DNA) and lies close to sites of free radical production by the process of oxidative phosphorylation, thus making it highly prone to oxidative damage. While there have been studies regarding the incorporation of 8-oxo-GTP and 2-hydroxy ATP by the multisubunit RNA polymerase from E. coli and human RNA polymerase II in to the growing RNA chain, the effect

of reactive oxygen species on the single subunit mitochondrial RNA polymerase particularly in plants has not been characterized to date. How AtRPOTm faces this challenge of oxidative stress in plant mitochondria is not known. In order to address these questions, the full length enzyme was cloned, expressed and purified and it was demonstrated that the purified enzyme is capable of performing transcription in vitro, whereas the catalytic mutant is inactive. The fidelity profile of the enzyme was also generated and its ability to incorporate oxidized nucleotides in RNA was also tested. Ongoing crystallization trials for the initiation and elongation complexes will reveal the structural details that underlie the mechanisms followed by this RNA polymerase in incorporating oxidised nucleotides into RNA.

Transcription regulation of flagellar gene network in Pseudomonas aeruginosa

Bacterial enhancer binding proteins (bEBPs) belong to AAA+ (ATPases 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 s54-RNAP to activate transcription. All bEBPs, like the eukaryotic transcriptional activators have a modular structure consisting of three distinct functional domains. The N-terminal domain serves as a target for regulatory signals. The C-terminal DNA binding domain consists of a helix-loop-helix motif and recognizes the upstream activating sequences. A central domain that interacts with the s54 is responsible for transcription activation and ATP hydrolysis. Although structures of various domains of s54-dependent activators and cryo-EM reconstruction of one of the activators bound to RNAP are available, information on the nature of conformational changes that occur in the activator upon RNAP binding is lacking. Most importantly, the allosteric mechanism that results in melting of promoter DNA bound to s54-RNAP on ATP hydrolysis by bEBPs is not known. In order to address these questions, the FleQ and FleR regulators of flagellar genes in Pseudomonas aeruginosa (Psa) were employed as a model system. FleQ and FleR control the expression of flagellar genes in a s54 dependent fashion and are class I and class II proteins respectively. FleR along with FleS together constitutes the two component signalling pathway. Deletion or mutation of fleQ or fleR genes makes the bacterium non-motile. In addition, fleR knockout makes the bacterium non-adhesive to mucins. Full length FleQ and FleR and their various domains were cloned, expressed and purified. Crystallization trials of various domain constructs and domain combinations alone or in complex with ATP or DNA were commenced. The central domain or the ATPase domain of FleQ has been crystallized in the apo state and in complex with a non-hydrolysable ATP analog. Diffraction data were collected upto 2.36Å resolution at the ID 29 beam line at European Sychrotron Radition Facility, France and the structure determined. The structure revealed that the FleQ central domain crystallizes as a hexamer and arranges in the form of helical bundles. Based on the structure, rationally designed mutants of the FleQ-central domain were cloned, expressed and purified and structures of the mutant proteins determined. This structural knowledge of the mutants will enable assessment of the ability of these mutants to regulate transcription through in vivo complementation assays. The interactions between activator and 54-RNAP will also be investigated. In addition, the complexes of regulatory factors with various RNAP subunits and cognate DNA sequences will be determined by macromolecular crystallography.

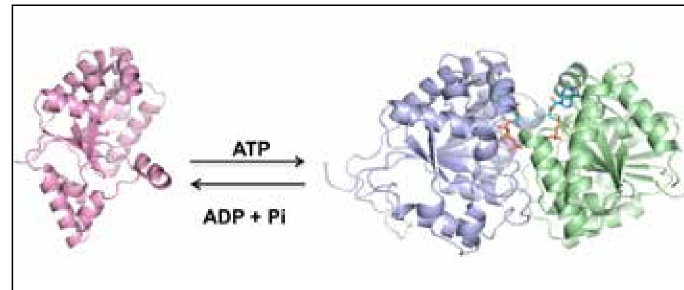

Figure 4: ATP mediated dimerization of the antiactivator FleN.

The activity of FleQ is in turn regulated by another protein FleN. FleN is a putative ATP/GTP binding protein that interacts directly with FleQ without affecting its DNA binding ability. Mutation of fleN results in a multiflagellated bacterium that shows chemotactic defects due to upregulation of flagellar genes. Thus, FleN acts as an anti-activator that regulates flagellar numbers by regulating the activity of FleQ. In order to obtain mechanistic insights into the modes of transcription regulation by FleN, the molecular interactions between FleQ and FleN are being studied. Overall, the studies reveal that ATP-induced structural remodeling facilitates formation of the functional dimer in FleN and helps the anti-activator attain a reversible form that can calibrate FleQ activity to an optimal level through a novel anti-activation mechanism (fig. 4). The process of validating the structural studies by in vivo experiments through complementation assays using the knockout of these proteins in Pseudomonas aeruginosa, as well as optimization of screening conditions for FleQ-FleN complex crystallization will be performed in the near future. Current efforts focus on investigating the interaction between the activator and the antiactivator (FleQ-FleN complex) through X-ray crystallography.

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

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 kinase and the receiver domain of the response regulator are widely conserved, as a result of which 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 Sensor Regulator) system is a typical two component system in Staphylococcus aureus, where VraS is the sensor histidine kinase and VraR is the response regulator. It was observed earlier that the inactivation of VraS resulted in increased tolerance to glycopeptide antibiotics like vancomycin, raising the possibility that the response regulator was being activated by an alternative kinase possibly GraS, which is a part of the GraSR (glycopeptide resistance associated Sensor Regulator) two component system. The study aimed to carry out a rigorous structural investigation of the interactions responsible for cross talk between the VraSR and GraSR regulon. In particular, the role of GraX in communicating the signals for cross talk between VraSR and the GraSR regulon were investigated. The graX, graS, vraG, vraR, and vraS genes were cloned into expression vectors. The graX knockout mutant strain of Sta was also prepared. In order to verify if GraX is the potential candidate for mediating the cross talk between the two two-component systems, the effect of this mutation was analysed using qRT-PCR with genes under both VraSR and GraSR regulons. The data showed that deletion of graX affects both the VraSR and GraSR genes. In addition, it was observed that the graX mutant was more prone to forming biofilms as compared to wt Sta. We have now crystallized The N-terminus domain of GraX was crystallized and diffraction data collected at the ESRF-ID23-1 beamline upto 2.5Å. A seleno-methionine derivative of GraX-NTD was also purified and crystallized and data collected upto 2.6Å resolution for determining the structure of GraX. Antibiotic resistance in S. aureus 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 responsible for the increase in glycopeptide tolerance in Sta.

In the future, the arsenal of tools to decipher details regarding the various interactions within the molecular assemblies will be expanded to include cryo-electron microscopy. Recently, a project on structure function analysis of non-structural proteins of the Chikungunya virus was also initiated, with the aim to exploit atomic level details for target identification through collaborative research for design of drugs against Chikungunya fever.

Structural Biology of Host-Microbial Interactions in Health and Diseases

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Microbial attachment to the host surface is the first and key step in colonization that may help or harm the host based on what kind of relationship the microbes develop with the host. The microbes often use their cell surface molecules to mediate attachment for establishing the contacts with their host. Developing drugs that can interfere with initial attachment to the host surface, and using good microbes to fight off the bad ones are seen as promising approaches in improving health and controlling infections. For these approaches, the knowledge of mechanisms by which the microbes assemble their surface molecules and use them for interaction with the host is essential. This programme conducts structural investigations on molecules that mediate interaction with the host from beneficial and harmful microbes. New insights have been obtained about how a probiotic bacterium assembles hair-like fibers called pili on its cell surface for colonization in the gut.

This research program aims to understand the structural biology of host-microbe interactions in health and disease. The present focus is on cell surface proteinaceous components that mediate initial attachment or interaction between the microbes and the host surface for colonization and biofilm formation. The major interest lies in visualizing host-microbial interfaces through structural biology tools at the atomic level towards understanding the mechanism by which microbes adhere to and interact with the host surface for colonization. Most of the later events in the pathogenesis or probiosis are highly dependent on the success of this primary interaction or adherence. Interfering with the host-microbial interface is considered as a promising approach for improving health and combating infections. Towards providing essential foundations for this approach, the study would generate structural knowledge of these interfaces by characterizing key molecules that establish the contacts between the host and microbes, both beneficial and pathogenic.

The host-microbial interface is constituted by myriad complex interactions, which depend largely upon the success of adhesion. Adherence involves specific complementary interactions between host receptors and microbial cell surface molecules (e.g. adhesins). This specific interaction likely defines host specificity and tissue tropism. However, attaching to the host surface is not a very simple task for microbes as they have to pass through multiple challenges including host physical and immunological clearances. To avoid being removed at the host surface, the microbes particularly bacteria, often assemble hair-like organelles known as fimbriae or pili on their cell surfaces for quickly and efficiently initiating adherence. Since the microbial surface adhesive molecules are immunogenic, they are also considered ideal vaccine candidates. As part of the above stated major goal, a structural investigation programme studies pilus constituents from beneficial and pathogenic strains for understanding the structural basis of pilus biogenesis, architecture and pili-mediated interactions. The study targeted some beneficial strains from gut microbiota as their knowledge is relatively limited compared to pathogenic strains. These organelles have been structurally studied to a great extent in Gram-negative pathogens while the Gram-positive pathogens have received attention only during the last decade. Few pathogenic members (e.g. primary colonizers of oral biofilm) have also been included in the study for getting insights into tissue tropism and understanding microbial interaction strategies in health and diseases.

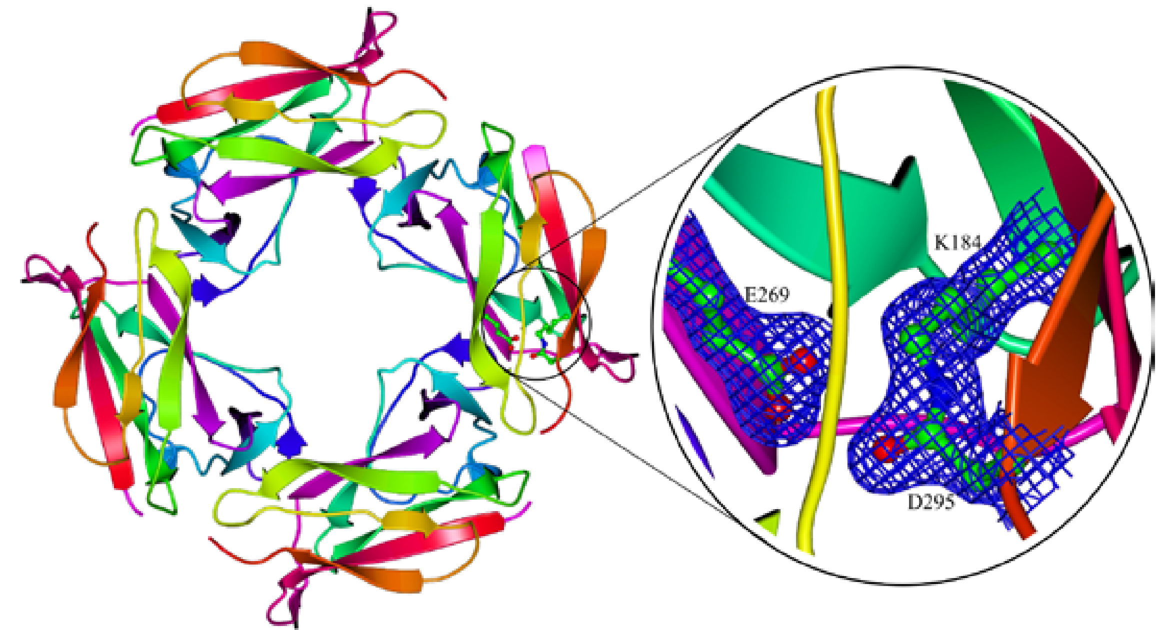

Figure 5: Crystal structure of C-terminal domain of SpaA showing isopeptide bond

Lactobacillus rhamnosus GG is a beneficial human gut microbiota isolate and a widely used probiotic because of its various health promoting effects. Its genome contains loci for two different pilus operons (spaCBA and spaFED) for sortase-mediated pili formation. The spaCBA operon encodes a major pilin (SpaA), two minor pilins (SpaB and SpaC) and a pilin-specific or C-type sortase (SrtC1). Similarly, the spaFED contains genes for a major pilin (SpaD), two minor pilins (SpaE and SpaF) and a C-type sortase (SrtC2). The pili in L. rhamnosus GG seem to be the major contributing factors in human gut adherence and colonization, and have a key role in persistence and immunomodulation for the beneficial health effects. Recent studies have shown that the L. rhamnosus GG utilizes pili to mediate interaction with intestinal mucus and components of extra cellular matrix (ECM). Towards understanding molecular mechanism(s) by which this bacterium assembles and adheres to host surfaces, structural investigation was begun for constituents of SpaCBA and SpaFED pilus including their respective sortases. A housekeeping or A-type sortase (SrtA), whose gene is located outside the pilus operon in the genome, covalently anchors the assembled SpaCBA, and SpaFED pili on the cell wall.

SpaA is a 35kDa backbone pilin in SpaCBA pili and its three-dimensional structure was earlier determined in the study at 1.9Å resolution using a combination of single anomalous dispersion (SAD) and molecular replacement (MR) phasing methods. It consists of two immunoglobulin-like CnaB (Collagen-binding adhesin B repeat fold) domains each with an isopeptide bond. The isopeptide bond is formed between lysine (K47) and asparagine (N172) in the N-terminal domain, and it is intriguingly formed between lysine (K184) and aspartate (D295) in the C-terminal domain with the respective catalytic glutamates E139 and E269 (fig. 5). Several mutants were constituted to analyze the effects of catalytic glutamates and isopeptide bonds on the structural stability and pilus polymerization. Subsequently, crystal structures of these mutants were solved and analyzed. Alanine substitution at E139 prevented the isopeptide bond formation with minor changes in conformation. However, the same substitution for E269 did not prevent isopeptide bond formation as well as pilus polymerization in contrast to existing knowledge on pathogenic strains. This substitution also enabled the capture of a snapshot of the truncated N-terminal domain with significant conformational changes. The crystal structure of the N-terminal domain provided insights about key roles of a flexible loop and C-terminal tail in stabilizing the structure, and formation of the isopeptide bond and the pilus fiber. Until publication of this structure, obtaining the crystal structure of labile N-terminal domain in pathogenic bacteria had been a challenge in the field. The proposed model based on the crystal packing for pilus formation also explained how this bacterium assembles an elongated spring-like fiber for withstanding shear forces faced during gut colonization. These structural analyses have provided new insights about pilus formation for the first time from a beneficial bacterium. To further understand the mechanism of isopeptide formation in the C-terminal domain, several substitutions were made at E269 position, and their crystal structure determination initiated.

SpaD is a 50kDa backbone pilin in SpaFED pili and consists of three immunoglobulin-like domains. The structure of C-terminal fragment containing two domains was initially generated by limited proteolysis. The crystal structure of this fragment was solved by iodide-SAD phasing. In the beginning, obtaining indexable

Figure 6: SpaC crystal

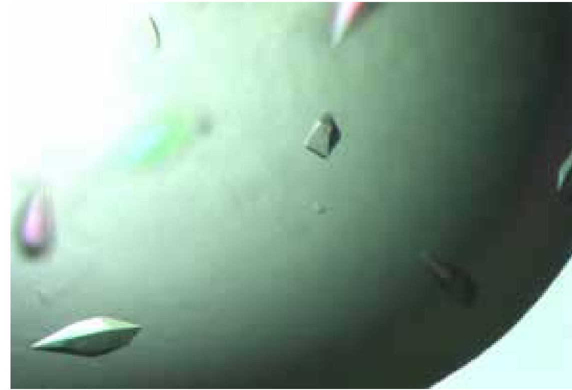

Figure 7: Crystals of SrtC1

crystallographic data from full-length SpaD crystals had been a challenge as their X-ray diffraction pattern was anisotropic with streaky spots. After optimizing the parameters in crystallization and diffraction experiments, indexable X-ray diffraction data upto 2.8Å resolution were collected from two different forms of crystals. Due to difficulties in modeling the flexible N-terminal domain, a selenium-derivative was later prepared for Se-SAD phasing. Crystal structures of the full-length SpaD have been obtained and its structural analysis is underway.

SpaB and SpaE are basal pilins in SpaCBA and SpaFED pili respectively. They were purified in soluble and pure forms. Promising initial hits were observed for both of these pilus proteins in crystallization experiments. Crystallization conditions for SpaE were further optimized to produce diffraction quality crystals. Trigonal form crystals produced using polyethylene glycol 3350 as a precipitant diffracted to 3.1Å resolution. Further optimization with various additives led to crystals of orthorhombic form, which diffracted to 1.5Å resolution. More recently, selenium-substituted SpaE crystals were grown and X-ray diffraction data were also collected at the peak wavelength of selenium for Se-SAD phasing. Model building and refinement for structure determination have been initiated.

SpaC is a 90kDa tip pilin and is primarily responsible for SpaCBA pili-mediated adhesion. It was crystallized earlier in the presence of magnesium ions and the X-ray diffraction data were collected to 2.6Å resolution. Initial attempts to solve the structure by heavy atom derivatization were unsuccessful, and a selenium incorporated SpaC was later produced and crystallized (fig. 6). Recently, diffraction data have been collected from these crystals for Se-SAD phasing, and model building commenced. The ongoing work clearly indicates the presence of vWFA (von Willebrand factor type A) domain with MIDAS (metal ion-dependent adhesion site) motif that likely facilitates interaction with host receptors. Hence, cloning of truncated vWFA domain has also been planned for examining the binding with various possible host receptors. The L. rhamnosus sortases have also been produced for crystallization and enzymatic analysis. Some initial crystallization hits were recently identified for SrtC1 (fig. 7).

Lactobacillus ruminis is a motile bacterium that attaches to the gut of human and other mammals. It contains pilus operon (lrpCBA), which encodes three pilins (LrpA, LrpB and LrpC) and one sortase (SrtC). The LrpCBA pilus type seems to be different from the SpaCBA and SpaFED pili of L. rhamosus GG, and represents a third sortase-mediated pilus type in Lactobacillus species. In contrast to SpaCBA and SpaFED pili, the LrpCBA lacks mucus-binding but shows affinity to collagen and fibronectin. This suggests that LrpCBA pilus structure and mechanism of interaction could be different from that of SpaCBA and SpaFED pili. Attempts are underway for producing the pilus components of LrpCBA to understand colonization strategy of L. ruminis, and how it differs from L. rhamnosus GG and pathogenic strains.

Similar to the gut, the oral cavity is also a habitat for multiple microbes that play a major role in oral ecology. Saliva keeps the oral ecosystem in balance by constantly bathing the mouth and flushing out bacteria. However, certain oral bacteria are able to stick to the surfaces of the oral cavity through their pilus adhesins and develop oral biofilms commonly referred to as plaque. The plaque damages teeth and gums, and leads to several periodontal diseases. The streptococci bacteria colonize the oral tissues immediately after brushing within few minutes for plaque formation. These species have become a focus of investigation, as they are the primary colonizers providing adhesion sites for secondary colonizers in oral biofilm development. Pilus components from these primary colonizers are being produced for structural and functional studies.

Pathophysiology of Thrombosis, Inflammations and Immune Responses in Various Disease Conditions

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This program is investigating the mechanisms for high risk of thrombosis and hypercoagulation in hemolytic disorders such as PNH and SCD; and the complex underlying reasons for inflammations and immune responses in these patients. Also under study is the phenomenon of drop in platelet counts in patients with dengue virus (DV) infections, and how the virus propagates rapidly during infections. The studies on mechanism of adaptation under extreme hypoxic (low oxygen) conditions at high-altitude show that certain gene mutations are associated with the adaptation of the native highland Tibetans. Further investigations explore how these gene mutations are associated with the protection against hypertension, edema and thrombosis; and against infections in these native highlanders.

The research programme is investigating the crosstalk between thrombosis/coagulation and inflammation/ immune hemostasis, perturbation of which develops clinical severity under different diseases conditions including hemolytic disorders such as Paroxysmal Nocturnal Hemoglobinuria (PNH) and Sickle Cell Disease (SCD), viral infections, high-altitude hypoxia-induced edema and Chronic Obstructive Pulmonary Disorder (COPD) and diabetes, Complement factor H related protein (CFHR1)-deficiency, and Small for Gestational Age (SGA) neonates.

Platelet pathophysiology contributes to inflammation and immune responses in patients with hemolytic disorders

Investigators in this group had recently described that hemoglobin (Hb) binding to GP1b on platelets surface induces platelet activation and in turn promotes pro-thrombotic/coagulation events in patients with PNH and SCD. Platelet activation directly correlates with their plasma level of free-Hb as well as platelet-surface bound Hb in these patients. Furthermore, another study from this program had shown that upon the intake of Hb-activated platelets the monocytes ($CD14CD16^-$) are transformed in large numbers, into pro-inflammatory subtypes ($CD14CD16^{high}$) in vitro and in vivo in the above patients. These observations have been further extended to assess the phenotype and function of neutrophils upon uptake of Hb or Hb-activated platelets, and the differentiation of monocytes into macrophages. Data show that the neutrophils are activated rapidly and form aggregates with other blood cells, and also adhere to the endothelium and trigger inflammation, when they engulf Hb-activated platelets. As shown in fig. 8, the hemolytic mice show elevated inflammation at the peritoneum cavity when LPS was injected at the site. The lavage fluid collected from the site of injury shows high amounts of activated neutrophils and neutrophil-platelet aggregates, as well as activated monocytes and monocyte-platelet aggregates. Another study from this group shows that the monocytes upon engulfing Hb-activated platelets are differentiated into pro-inflammatory M1 macrophages. On the other hand, the monocytes that engulf only Hb are differentiated into M2 or anti-inflammatory macrophage subtypes.

Platelet activation contributes to thrombocytopenia in dengue virus infection, and the platelet secretome promotes dengue virus propagation

In recent work, the study has shown that the activation stages of platelets correlate directly with platelet destruction by dengue virus (DV) in vitro. The high

copy numbers of DV genome in platelets directly correlate with platelet activation during day 4, the early day of fever, and decrease during day 10 in patients with DV infections. The DV-activated platelets are rapidly engulfed by monocytes and macrophages, and get cleared from circulation. Investigations into the role of platelet proteins (which are up taken by phagocytes) in the rapid replication of DV in monocytes or other phagocytic cells during initial days (day 1-5) of infections in patients are also ongoing. It is known that phagocytic cells engulf DV, and that DV utilizes these host cells for replication and propagation. Data show that DV replication increases significantly in monocytes that have taken up DV-activated platelets. Further, this study is investigating the mechanisms as well as platelet proteins that promote DV replication in monocytes in patients.

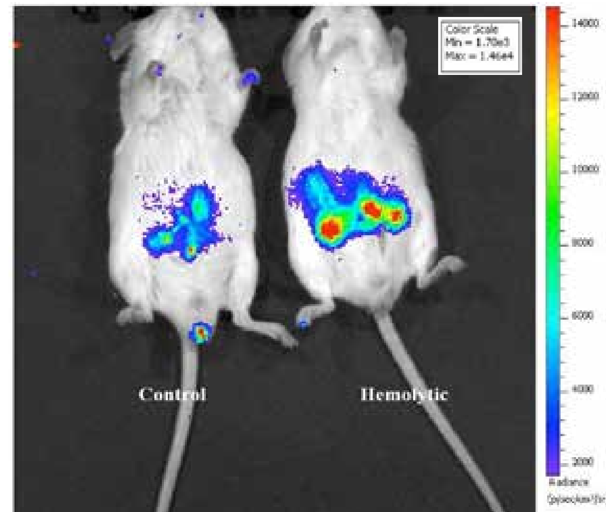

Figure 8: Mice with intravascular hemolysis exhibit higher inflammation when injected with LPS. 4-6 week old BALB/c mice were injected (i.p) with 1) phenylhydrazine (1 mg/25g body wt) to induce intravascular hemolysis, or 2) vehicle buffer (to control). After 24-36 hrs, a single dose of LPS (i.p; 100 g/25g body wt) was injected at peritoneum to induce local injury. The above image was captured after luminol injection (i.p; 2mg/25g body wt) after 3hrs of LPS treatment using in vivo imaging system (IVIS Spectrum In Vivo Imaging System, Parkin Elmer). The image shows increased amount of myeloperoxidase in hemolytic mice (luminol interacts with the myeloperoxidase, induces fluorescence).

Native highlanders with genetic adaptations show protection against hypercoagulation, inflammations and edema

As an extension of recent findings by this group describing novel mutations in hypoxia responsive gene EGLN-1 (C12G and G380C) and their association with decreased erythropoiesis in native Tibetans at high altitude, further investigations into the association of these polymorphisms with events such as hypercoagulation, inflammations and edema among the native highland (Leh) Tibetans with and without the above mutations were undertaken. The study is analyzing polymorphisms and their association with the profiles of coagulation factors, inflammatory cytokines and immune cells. Besides, monocyte cell lines with EGLN-1 (C12G and G380C) mutations have been generated for investigating the role the above mutations on the phenotype and functions of this immune cell including cytokine secretion, tissue factor secretion and phagocytosis.

The crosstalk between CFH and CFHR regulates immune tolerance in HUS patients as well as healthy individuals

The complement factor-H related protein domain-1/3 (CFHR1/3) deficiency is one of the risk factors for many autoimmune diseases such as atypical hemolytic uremic syndrome (aHUS), systemic lupus erythematosus (SLE), C3 glomerulopathy, IgA nephropathy and rheumatoid arthritis (RA). These patients exhibit strong association between homozygous deficiency of CFHR1/3 and the presence of autoantibodies against complement factor H (CFH), which is one of the major regulatory proteins of the complement pathway. The cause of this breakdown in immune tolerance remains unknown. Interestingly, the CFHR1/3 homozygous deletion appears as a polymorphism at varying frequencies in healthy individuals of different ethnic groups. In India, 8-10% healthy individuals are CFHR1/3 null. The study therefore investigated the role of CFHR1 and 3 proteins in the modulation of the immune response(s). The data show that the CFHR1/3 null individuals have higher frequencies of plasmacytoid dendritic cells, patrolling monocytes and non-Treg CD25+CD4 T cells and higher surface bound CFH levels on classical monocytes with no difference in cell surface-associated C3b. Monocytes from CFHR1/3 null individuals were more responsive to Toll Like Receptor (TLR) ligands. These differences collectively suggest a possible role of CFHR 1/3 proteins in the modulation of immune response(s).

Altered responses of monocytes and lymphocytes increase susceptibility towards infections in neonates with SGA

To assess the impaired immunity of the SGA neonates, the study is immunophenotyping (particularly monocyte, T cell and B cell subsets) the cord blood from SGA and appropriate for gestational age (AGAs) neonates and comparing with adult immune phenotypes. The initial data show that SGA neonates have comparatively fewer plasmacytoid DCs (pDC), a higher myeloid DC to pDC ratio and more natural killer cells in cord blood. Besides, the susceptibility of the SGA neonates towards infections is also being investigated. To understand better the development of the immune system in neonates, analysis of the above data of cord blood immune phenotypes of SGA and AGA neonates in correlation with the immune signatures of the adults is also being performed.

Biology of Medically Important Viruses

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Infectious diseases pose an ever increasing threat to the well-being of human population at large and this scenario is particularly precarious in the Indian context where epidemics of various viral infections are reported at regular intervals. Understanding the biology of virus infection and replication can help in designing novel antivirals for effective therapeutic and prophylactic interventions. This program aims to study the various proteins of medically important viruses to help design the novel antiviral molecules.

Outbreaks of Chikungunya virus (CHIKV) are being reported from the various parts of India and there is no virus-specific treatment available. This program aims to identify potential antiviral molecules by understanding the structure and function of the important viral proteins. To this end, researchers are aiming to express and purify the E1, E2, NSP1 and NSP4 proteins of CHIKV and study their biology. In silico or in vitro methods will then be used to identify small molecules that may interfere with a critical biological function of these protein thereby inhibiting the virus infectivity.

Studies on CHIKV E1 and E2 proteins with host cell receptor/s

Host cell receptor recognition by CHIKV is the first step in infection. The envelope-anchored spike proteins (E1 & E2) mediate CHIKV entry into the host cells first by binding to the cell surface receptor/s and then by fusing into the host membrane. To elucidate the mechanism of attachment and fusion by investigating the CHIKV-host interactions at the atomic level, two approaches involving in silico and in vitro experiments were adopted. For the in silico experiments, the predicted host-cell receptors from the published studies have been identified and chosen for the initial interaction studies. The structures of host receptors and CHIKV envelope proteins were retrieved from the Protein Data Bank (PDB). The structural models were prepared and subjected to molecular-docking. Representative poses from the top ranked clusters were chosen for structural analysis. The hotspots comprising key surface residues involved in the interaction were identified. Screening of library of compounds that could disrupt the interactions is in progress. Additional receptor candidates will be included in the docking studies in the next phase. For in vitro experiments, E1 and E2 proteins have been expressed in E. coli and expression in insect/mammalian cells is underway to produce soluble proteins for interaction studies. The purified protein samples will be used in biophysical binding studies using surface plasmon resonance (SPR) for cross-validating the in silico experimental results, and narrow down the effective compounds. The structural investigation of envelop protein-receptors complex will be aimed for studying mode of interaction and mechanism of inhibition.

Studies on CHIKV NSP4 protein

The NSP4 protein of CHIKV houses the RNA-dependent-RNA polymerase activity and is centrally involved in replication of its RNA genome of this Alphavirus. The structural mechanism utilized by NSP4 from CHIKV or

any other Alphavirus to achieve function is not known. To carry out structural and biochemical studies on CHIKV NSP4, a codon-optimized synthetic cDNA construct of the full-length enzyme has been obtained. Based on the bioinformatics analysis, four different constructs of this enzyme were cloned into two different expression vectors and the expression of these constructs is being tested in various bacterial expression strains. It was seen that in the strains tested so far, the constructs are expressed but are found in the pellet fraction due to aggregation. For the constructs tagged with a 6-His tag, different refolding protocols in the presence of different additives are currently being tested to obtain soluble protein. For one of the constructs, a protocol involving rapid dilution of the denaturant using refolding buffer appears promising and may yield soluble protein. Once soluble protein is obtained, primer extension assays will be carried out to assess activity and crystallization trials will be carried out on the enzyme in complex with substrate RNA and incoming nucleotide.

To define the CHIKV replication complex, components of the viral and host proteins interacting with NSP4 are being identified using a mass spectrometry-based interactomics approach. CHIKV NSP4 was cloned into a mammalian expression vector to generate a construct with a His-FLAG-SBP tag to the protein. The correctness of the clone was confirmed by DNA sequencing. This construct (NSP4-MTAP-mVenus) was transfected into U20S (human osteosarcoma) cells. Subsequently, stably integrated transgenic cells were selected in the presence of hygromycin. Expression of the NSP4-fusion protein in U2OS cells was confirmed by Western blotting analysis. The NSP4-expressing stable transgenic cell line will now be used for identification of essential host-viral protein interacting partners by mass spectroscopy.

Studies on CHIKV NSP1 protein

NSP1 is the primary enzyme containing methyltransferase and guanylyltransferase activity and is involved in RNA capping in CHIKV. The protein adds covalently attached cap moiety at the 5′ end of the RNA to protect the viral genome from degradation by exonucleases and for efficient translation. The capping mechanism of NSP1 is novel and unlike in humans involves methylation of N-7 atom of GTP, covalent attachment of m7-GMP to NSP1 followed by transfer of this adduct to 5′ end of viral RNA. The project involves structure function analysis of NSP1 which is a potential target for anti-viral therapy. The expression and purification of the full length NSP1 using different constructs and expression hosts has been optimized. The purified enzyme is being tested for its activity. The purified enzyme is prone to degradation. More stable constructs of NSP1 that are suitable for structural analysis are being designed through limited proteolysis, N-terminus sequencing and mass-spectrometry. The more stable constructs will be cloned, purified and will be subjected to structural analysis. Snapshots of different stages of capping mechanism will be obtained through the crystallographic tools.

Post-Translational Protein Modification: Involvement in Cellular Processes and Disease Regulation

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Protein modifications serve various purposes in a wide variety of cellular processes. Identification, characterization, and mapping of these modifications to specific amino acid residues on proteins are critical towards understanding their functional significance in a biological system and disease regulation. The major goal of research in this program is to understand how these protein modifications alter the neurodegenerative conditions like Parkinson's disease, Alzheimer's disease and different type of cancers.

This program aims to understand how post-translational modifications of proteins regulate the diverse cellular signalling events and their mechanism of disease regulation. Protein post-translational modifications (PTM) such as phosphorylation, ubiquitination, SUMOylation, redox-modification, acetylation and glycosylation play important roles in different cellular processes including protein quality control, cell cycle regulation, endocytosis, DNA repair, vesicle trafficking and others. Dysregulation of these processes leads to diseases like cancer.

One of the important aspects of this research program is to understand the ubiquitin signaling mechanisms and their regulation in cellular pathways and disease. Human genome analysis and proteomics data reveal almost one hundred deubiquitinating enzymes (DUBs), which majorly regulate ubiquitin homeostasis in cells. However, molecular functions of most of the DUBs are still elusive. The molecular basis of the involvement of DUBs in cellular functions like protein degradation, histone modification and endocytosis of plasma membrane proteins is being investigated. It has also been revealed that the dysregulation of deubiquitinating enzymes leads to diseases like Parkinson's, Alzheimer's, ataxia, heart disease and different types of cancer. The aim is to understand the possible molecular mechanisms underlying these diseases. Another focus of the program is to understand how redox modification particularly Cys-nitrosylation regulates diverse cellular processes including disease outcomes from neurodegenerative disease to microbial infection.

Molecular insights in mutational inactivation of deubiquitinating enzymes in cancer

Over the last three decades, considerable progress in the field of genome sequencing has revealed the genomic landscape of cancer. Advancement in genomic studies has shown that there are more than a hundred genes altered due to intragenic mutation. These mutations are essential for oncogenic progression. In a specific tumor type, there are some driver genes that regulate core cellular processes like cell fate, cell survival and genome integrity. BRCA1 associated protein 1 (BAP1) is a nuclear deubiquitinase that regulates tumor suppressor activity and widely involves many cellular processes ranging from cell cycle regulation to gluconeogenesis. Impairment of enzymatic activity and nuclear localization induce abnormal cell proliferation. It is considered to be an important driver gene, which undergoes frequent

mutations in several cancers. However, the role of mutation and oncogenic gain of function of BAP1 is poorly understood. The cellular localization, enzymatic activity and structural changes of point mutants of the catalytic domain of BAP1 prevalent in different types of cancer were investigated. These mutations trigger cytoplasmic/ perinuclear accumulation of BAP1, which has been observed in proteins that undergo amyloid aggregation in cellular condition. It was demonstrated that mutations in the catalytic domain of BAP1 initiate structural destabilization that eventually produces beta amyloid structure. Overall, the results unambiguously demonstrate that structural destabilization and subsequent aggregation abrogate its cellular mechanism leading to adverse outcomes.

The USP family deubiquitinase CYLD, which specifically cleaves lysine 63-linked ubiquitin chains regulates a myriad of important cellular events including cellular differentiation, DNA repair, T cell development and activation, cell cycle regulation and oncogenesis. CYLD, which is one of the important tumor suppressor genes, down regulates NFkB and JNK signaling pathways. It deubiquitinates TRAF2/TRAF6, NEMO, Bcl3 and AKT and regulates many cellular events. Its role in host pathogen interactions has also been extensively studied. Germline mutations in CYLD are associated with a rare, hypertrophic skin cancer, termed familial cylindromatosis. Loss of CYLD in different cancers contributes to oncogenesis via its effects on NF-kB activation. The Catalogue of Somatic Mutations in Cancer database enlists CYLD point mutations in multiple benign and malignant tumors. The present study demonstrated that mutation in the CYLD catalytic domain exposes its hydrophobic core to the surface and impairs its substrate binding. The mutations perturb its catalytic activity, which is essential for its tumor suppression function (fig. 9).

Role of OTUB1 in neurodegenerative diseases

Neurodegenerative diseases that primarily affect neuronal cells are incurable and debilitating conditions that result in progressive degeneration and/or death of nerve cells. The neurodegenerative diseases include Alzheimer's Disease (AD), Multiple System Atrophy (MSA), Parkinson's disease (PD), Prion disease, Ataxia and Amyotrophic Lateral Sclorosis (ALS). AD and PD comprise almost 70 % of all neurodegenerative diseases. PD is the second most common neurodegenerative disease that is characterized by loss of dopaminergic neurons in the substantia nigra pars compacta. Majority of PD cases reported are sporadic. However, almost 10% of PD cases are familial in nature. Several genes have been identified in familial PD among which -synuclein, PINK1, Parkin, DJ-1 and LRRK2 have been well studied. Clinical as well as experimental observations support the hypothesis that increased expression of -synuclein is a cause for PD pathogenesis and increased cytoplasmic expression of -synuclein in aged human brain is one of the major risk factors for PD development. Genome wide association studies reveal that single nucleotide polymorphisms associated

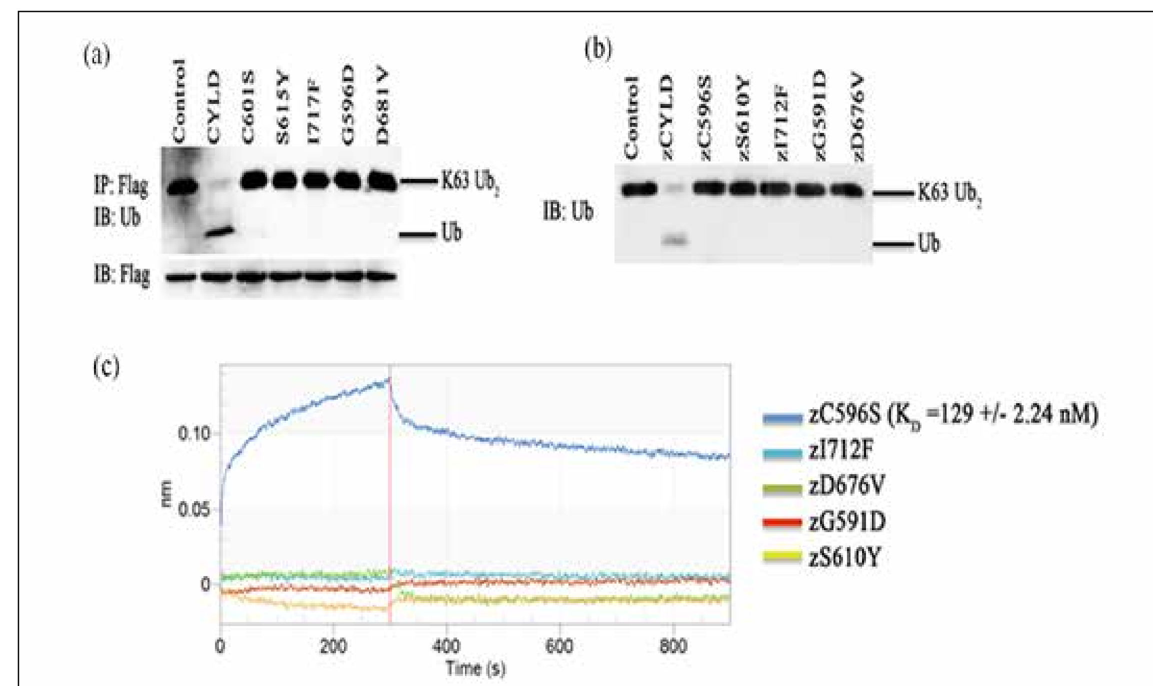

Figure 9: In vitro deubiquitination assay showing differential catalytic activity of CYLD wild type and its mutants against cleavage of K63 diubiquitin chain into ubiquitin (a) Human CYLD (b) Zebrafish CYLD^{Bbox} (c) K63 binding analysis with 10mM CYLD-wild type and its mutants with diubiquitin K63 chain fitted in 2:1 model.

with -synuclein are linked to increased risk of PD. PD pathology is characterized by the formation of the Lewy body (LB) which consists of several misfolded proteinaceous aggregates.

Oxidative stress, gene alteration, endoplasmic reticulum (ER) stress and dysfunction of ubiquitin proteasomal system lead to impairment of homeostatic regulation of protein quality control with a consequent increase in protein misfolding. The cellular ubiquitin pool is maintained by synchronous activity of ubiquitination and deubiquitination. There are several DUBs including USP8 and USP9X involved in PD that function by regulating the level of -synuclein via the deubiquitinase activity in conjunction with Ubc13 and NEDD4. Besides these DUBs, a novel deubiquitinating enzyme OTUB1 has been found by mass-spectrometry studies in the LB as well as in Alzheimer's plaques in patients. OTUB1 belongs to the class of cysteine proteases and comprises of 271 amino acids. It contains an OTU domain ranging from amino acid residues 80-271 that possesses catalytic activity. It is ubiquitously expressed in human tissues including brain, liver, spleen, lung, kidney, uterus, ovary, thymus and other tissues. The function of OTUB1 in cancer is well explored and it has a crucial role in several types of cancer. A recent study has demonstrated that OTUB1 attenuates neuronal apoptosis after intracerebral haemorrhage. It is also involved in Tau pathology by inhibiting the degradation of Tau protein that results in accumulation of Tau in brain.

Mass spectrometry based proteomics data analysis of the Lewy body identified that OTUB1 is enriched along with -synuclein. However, its role in Lewy body pathology and how this deubiquitinating enzyme accumulates in PD condition remain elusive. Here, the study conducted investigations to obtain molecular insight into the presence of OTUB1 in LB. The study investigated whether OTUB1 itself aggregates in vitro in heat induced conditions. The ThT and congo red binding results confirm that OTUB1 produces amyloid aggregates in heat induced conditions. Upon heating at 37°C, the alpha-helical component of OTUB1 gradually transforms into beta sheet structure and initiates oligomerization. Incubation at 37°C for 48 h under constant stirring condition produces a mature SDS-insoluble fibril. The oligomers and mature fibrils were characterized by high-resolution atomic force microscopy (fig. 10). It was discovered that OTUB1 oligomers as well as mature fibrils show cellular toxicity. The cellular toxicity of the OTUB1 oligomer is greater than that of the mature fibril as observed for many amyloid proteins. Cross seeding experiments demonstrated that OTUB1 effectively accelerates the fibril formation of -synuclein in a concentration dependent manner. To cross check whether -synuclein and OTUB1 localize in rotenone-induced cellular conditions, SHSY-5Y cells were treated with rotenone and immunofluorescence experiments performed. It was observed that OTUB1 accumulates in rotenone treated conditions but not in control cells. Moreover, OTUB1 co-localizes with -synuclein and ubiquitin in rotenone treated cells (fig. 11). These results indicated that there could be possible cross talk between OTUB1, -synuclein and ubiquitin and it may provide a new molecular mechanism in PD pathology.

Nitric oxide signaling determines cell death in Parkinson's Disease

Protein oxidative modifications comprise a major class of protein post-translational modifications. They are caused by reactions between protein amino acid residues and reactive oxygen species (ROS) or reactive nitrogen species (RNS). There are many events inside a cell that generate ROS/RNS that are useful at certain concentrations but show abnormal effect at increased concentrations. Mitochondria are recognized as the major site for ROS and RNS production and both complexes I and III have been established to be the specific sites for mitochondrial redox species generation. Nitric oxide (NO) is one such redox species acting as a gasotransmitter that plays a pivotal role in a variety of signal transduction pathways that are crucial for maintaining the physiological functions of vascular, respiratory, immune, muscular, and nervous systems. However dysregulated production of nitric oxide causes aberrant physiological processes leading to various types of diseases including neurodegenerative diseases. The serum nitric oxide level in Parkinson's disease patients is found elevated compared to control subjects. Therefore, global nitrosoproteome identification was

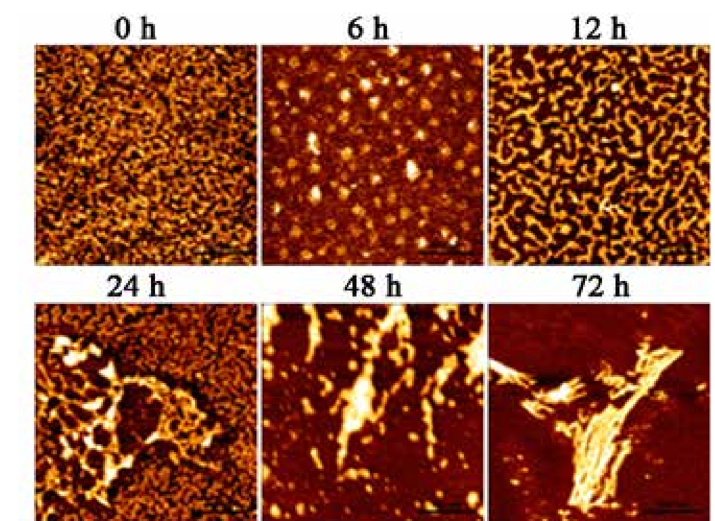

Figure 10: Surface topology of OTUB1 ordered aggregates at different time points. Images are in 2×2 μm size and scale bar is 500 nm.

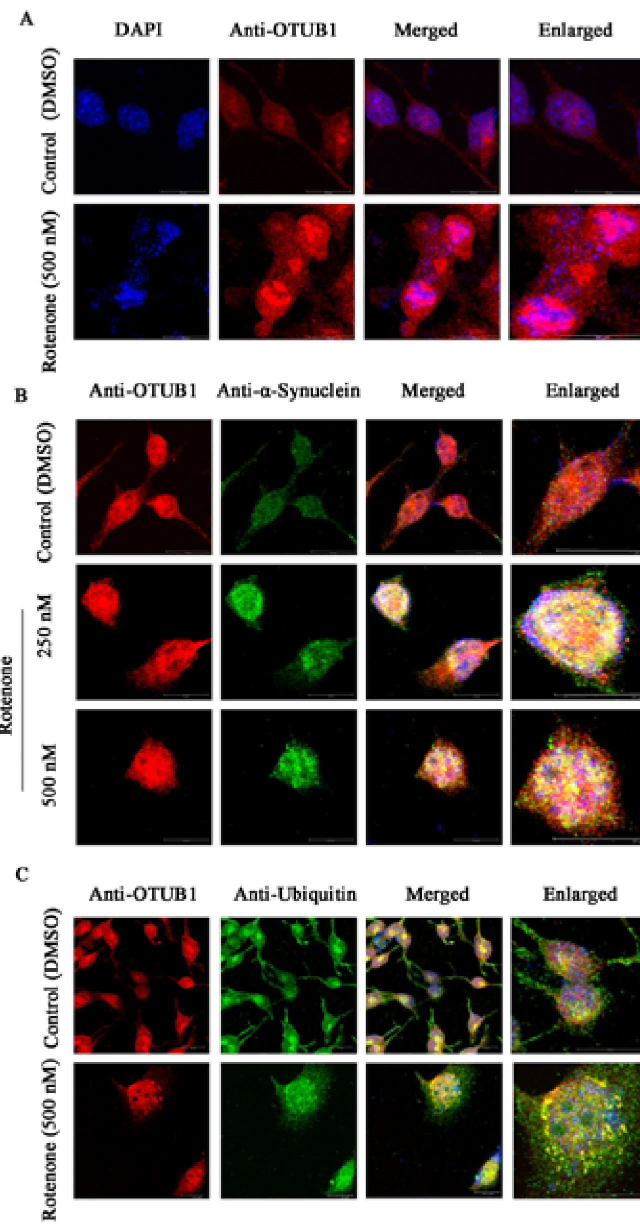

Figure 11: Effect of rotenone on OTUB1 in neuroblastoma SHSY-5Y cells. (A) Confocal images showing aggregates of OTUB1 in rotenone treated (500 nM for 48 h) condition. (B) OTUB1 co-localization with -synuclein in rotenone treated (250 nM and 500 nM for 48 h) condition. (C) Co-localization of OTUB1 with ubiquitin in rotenone treated (500 nM for 48 h) condition. Scale bar is 20 μm.

performed in a rotenone induced PD mouse model and rotenone treated SHSY-5Y cells. Mass spectrometry analysis of rotenone induced mice and SHSY-5Y cells identified 249 nitrosylated proteins. The functional annotation and clustering identify Ubiquitin C-terminal Hydrolase-1 (UCHL1) as an important nitrosylated protein that regulates protein catabolic processes, cognition, learning and memory. UCHL1 has been known to be associated with Parkinson's disease pathology for many years and many mechanisms have been proposed. However, the precise mechanism by which UCHL1 is associated with PD pathology remains unclear. The present study reported that UCHL1 undergoes S-nitrosylation in vitro and in a rotenone induced PD mouse model. S-nitrosylation of UCHL1 induces structural instability and produces amorphous aggregates, which in turn provide nucleation to the native -synuclein for faster aggregation. The results provided a new link between UCHL1-nitrosylation and PD pathology.

-Synuclein produces various pathogenic oligomeric species in PD condition that are secreted out of the cells. The extracellular -synuclein oligomers are internalized by other cells via different methods. Upon uptake, the oligomers interact with cellular membranes and impair the membrane structure and form membrane pores. The -synuclein membrane interaction and pore like structure have been shown in model membranes. The cell membrane damage and pore formation have also been shown by electrophysiology and fluorescence recovery after photo-bleaching (FRAP) studies. There is however no evidence of a nanoscopic view of cell membrane damage and downstream signaling events induced due to membrane perturbations by -synuclein. This study demonstrated the membrane damage and pore formation by -synuclein using atomic force microscopy (fig. 12). Elevated levels of nitric oxide have been observed in the cells with treatment of oligomeric species which resulted in S-nitrosylation of key proteins like Actin, DJ-1, HSP70, UCHL1, Parkin, and GAPDH that alter the cytoskeletal network, protein folding machinery, ubiquitin proteasome system and apoptosis, thus contributing to neuronal cell death which induces PD. Based on the study, -synuclein oligomers can be divided into severely damaging and less damaging categories. These severely damaging oligomers include wild type oligomer, A53T oligomer and iron induced oligomer and have strong membrane perforating ability because of their size and -content. The cold induced oligomer and wild type monomer are considered less damaging -synuclein species. Each species causes nitric oxide release to different extents and influences PD progression. In summary the current findings link redox stress with progression of PD pathology.

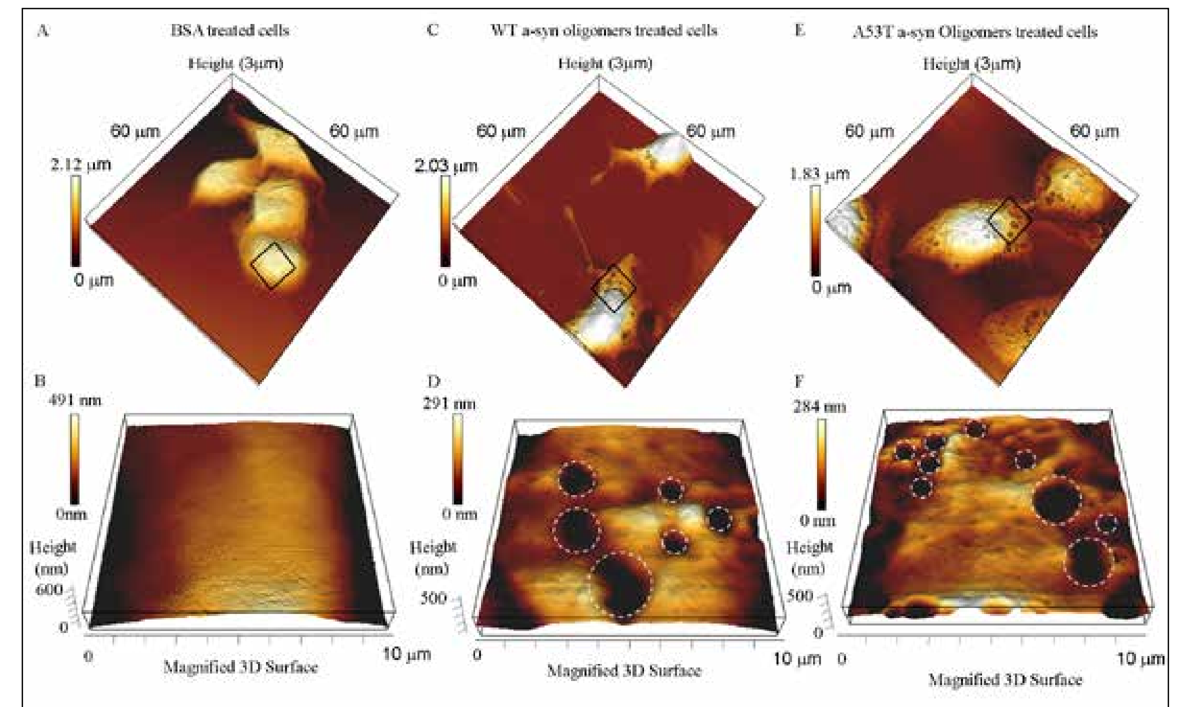

Figure 12: Differential membrane perforation induced by diverse types of -synuclein oligomers. Top horizontal panel represents 3D atomic force microscopic images (60 μm x 60 μm) of SHSY-5Y cells. Lower panel represents magnified 3D surface (10 μm x 10 μm). (A, B) BSA (C, D) wild type -synuclein oligomer (E, F) A53T -synuclein oligomer. The membrane damage is marked with white circle. Each image is presented with height scale. Distinct pore like features were clearly seen on neuroblastoma SHSY5Y cells treated with wild type and A53T -synuclein oligomer but were absent in BSA treated cells.

The mechanistic details of the BAP1-ubiquitin interaction remain enigmatic as the crystal structure of BAP1 has not been solved yet. Based on sequence similarity and homology modelling, it is predicted that the overall folding pattern of the BAP1 catalytic domain is similar to other UCH members. The dynamic nature of BAP1 suggests nuclear magnetic resonance spectroscopy (NMR) as an attractive method to reveal both its deubiquitinase function and interaction with ubiquitin. Mass spectrometry based interactome analysis demonstrated that BAP1 is co-enriched with heat shock proteins (Hsps) leading to the hypothesis that BAP1 interacts with HSP90 via its coiled coil domain to stabilize itself and avoids aggregation. To check which domain of HSP90 interacts with BAP1, domain wise truncations in BAP1 and HSP90 will be generated. Interactions will be tested and validated using FLAG pull down and surface plasmon resonance (SPR) interaction studies.

It was found that loss of CYLD shows a significant upregulation in the expression profiles of BiP and subsequent signaling cascade markers. These observations lead to the hypothesis that CYLD being a tumor suppressor gene is responsible for controlling various cellular responses through the Unfolded Protein Response (UPR) pathway, which needs to be elucidated further.

- Synuclein, apart from inducing membrane nanopores, also exerts mechanical force over cellular architecture and regulates PD pathology. To understand mechano-transduction induced PD progression in detail, an oligomer-infused PD mice model would be developed. The mechanical changes in mice brain using nano indent atomic force microscopy with gradual development of PD will be tracked. As earlier discussed, OTUB1 is present in the Lewy body and also forms amyloid aggregates. These clues prompt investigations into the molecular mechanism behind involvement of OTUB1 in Parkinson's disease. For in vivo studies, the rotenone induced PD mouse model would be generated to study the Parkinson's disease condition and to investigate the cross-talk between OTUB1, -synuclein, ubiquitin and Ubc 13.

Signals that Regulate Skeletal Muscle Structure and Function

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The skeletal muscle is an important tissue, crucial for mobility, posture, and support. To appreciate how the skeletal muscle performs its functions and how muscle diseases arise, a better understanding of proteins that help in muscle function is necessary. One muscle protein essential for muscle contraction is myosin. A myosin called myosin-embryonic is expressed during animal development with unknown functions, although mutations in it cause muscle disease. Using mouse models, this study finds that myosin-embryonic is important for skeletal muscle development and that its absence causes muscle defects, which should help gain a better understanding of human muscle diseases and develop new treatment strategies.

This program aims to understand the mechanisms underlying the process of cellular differentiation and its regulation. This is addressed using the skeletal muscle, focusing on skeletal muscle development, differentiation, stem cell-mediated regeneration, and signaling events in cancers that exhibit skeletal muscle characteristics, employing in vitro and in vivo approaches. The major goal is to understand the molecular mechanisms underlying skeletal muscle differentiation and how aberrant differentiation leads to skeletal muscle abnormalities and diseases. Towards achieving this broad aim, the expression dynamics and function of developmental Myosin Heavy Chains (MyHC) such as Myosin Heavy Chain-embryonic (MyHC-emb) in skeletal muscle development and differentiation are being characterized in vivo using mice, during embryonic and fetal stages. Investigations into the role of MyHC-emb during in vitro differentiation using C2C12 mouse myogenic cells are also being pursued. In addition, the expression and regulation of MET signaling in Rhabdomyosarcoma (RMS), a cancer type where the cells exhibit characteristics of differentiating muscle cells are being studied.

Myosins are motor proteins essential for cellular processes such as motility, division and transport of cargo. Among the different classes of myosins, one of the most important are the Class II myosins which comprise myosins critical for skeletal muscle contraction. Skeletal muscle contractile myosins are heterohexamers, comprising a pair each of Myosin Heavy Chains (MyHCs), Myosin Essential Light Chains and Myosin Regulatory Light Chains. This study pertains to the MyHCs to understand their expression dynamics and specific roles in skeletal muscle development, differentiation, regeneration and disease.

Multiple MyHC isoforms are present in mammals, mostly as part of the adult muscle contractile network. Different adult MyHC isoforms exhibit unique contractile properties and their expression in specific muscles is dependent upon functional demand; for instance, postural muscles used for long durations generally have higher expression of slower MyHCs, which contract slower, utilize oxidative metabolism and are relatively fatigue resistant. In addition to adult MyHC isoforms, three MyHC isoforms, namely MyHC-embryonic (MyHC-emb), -perinatal (MyHC-peri) and -slow, collectively known as developmental MyHCs, are expressed by differentiating muscle cells during embryonic development. Of these, two, namely MyHC-emb and -peri are exclusively expressed during embryonic stages, while MyHC-slow is expressed during embryonic development and adult life. MyHC-emb and -peri are re-expressed during adult life upon skeletal muscle injury or disease and subsequent muscle stem cell-mediated regeneration. Mutations have been identified in all of these MyHCs that cause congenital diseases such as myopathies and contracture syndromes like Freeman-Sheldon syndrome. Therefore, it is important to characterize the precise mechanisms underlying the regulation of expression and function of developmental MyHCs.

This study is characterizing the expression dynamics of developmental MyHCs during the course of mouse development. In order to do this, immunofluorescence using MyHC antibodies followed by confocal microscopy is used to detect MyHC proteins. Previous studies on vertebrate myogenesis indicate that two phases of myogenesis occur during development: embryonic myogenesis and fetal myogenesis. In mice, embryonic myogenesis spans from embryonic day 10.5-12.5 (E10.5-12.5), while fetal myogenesis occurs during E14.5-17.5. Mouse embryos of different developmental stages were harvested, fixed, embedded in cryo-protectant medium, sectioned using a cryomicrotome, and MyHC proteins detected by immunofluorescence using antibodies against specific MyHCs. At E10.5, MyHC-emb and -slow protein can be detected by immunofluorescence in the dermomyotome of the somites, where myogenic precursors originate (fig. 13). However, at E10.5, MyHC-peri protein is undetectable, suggesting that it is the last of the developmental MyHCs to be expressed during mouse embryonic development (fig. 13).

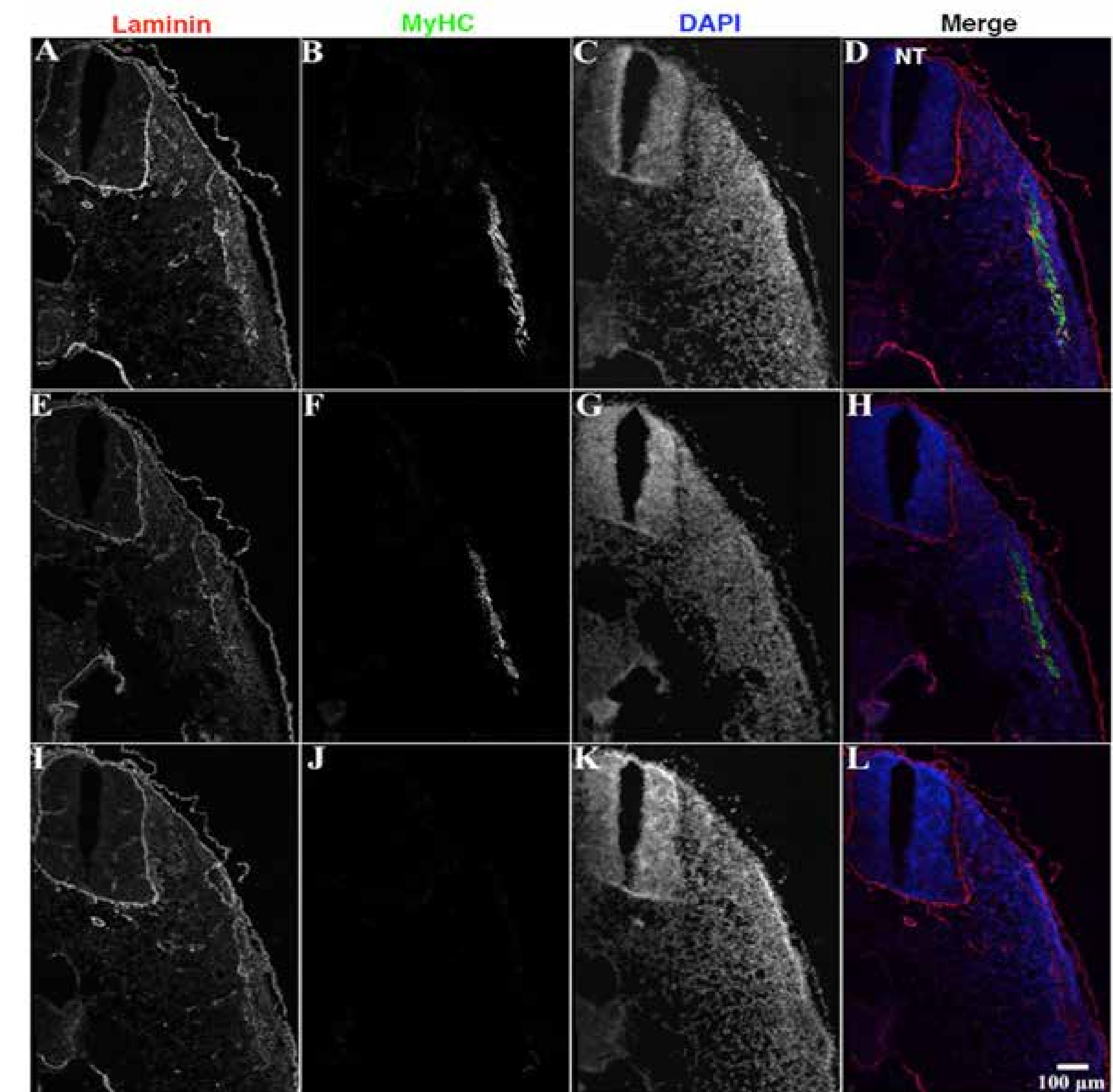

Figure 13: Developmental Myosin Heavy Chain (MyHC) expression at embryonic day 10.5 of mouse development. Confocal maximum projections of adjacent sections through an embryonic day 10.5 (E10.5) mouse embryo stained for Laminin (A, E, I), MyHC-emb (B), MyHC-slow (F), MY32 for MyHC-peri (J), DAPI for nuclei (C, G, K), and merge (D, H, L). The neural tube is marked (NT), and expression of MyHC-emb and to a lesser extent MyHC-slow is apparent in the somitic dermomyotome.

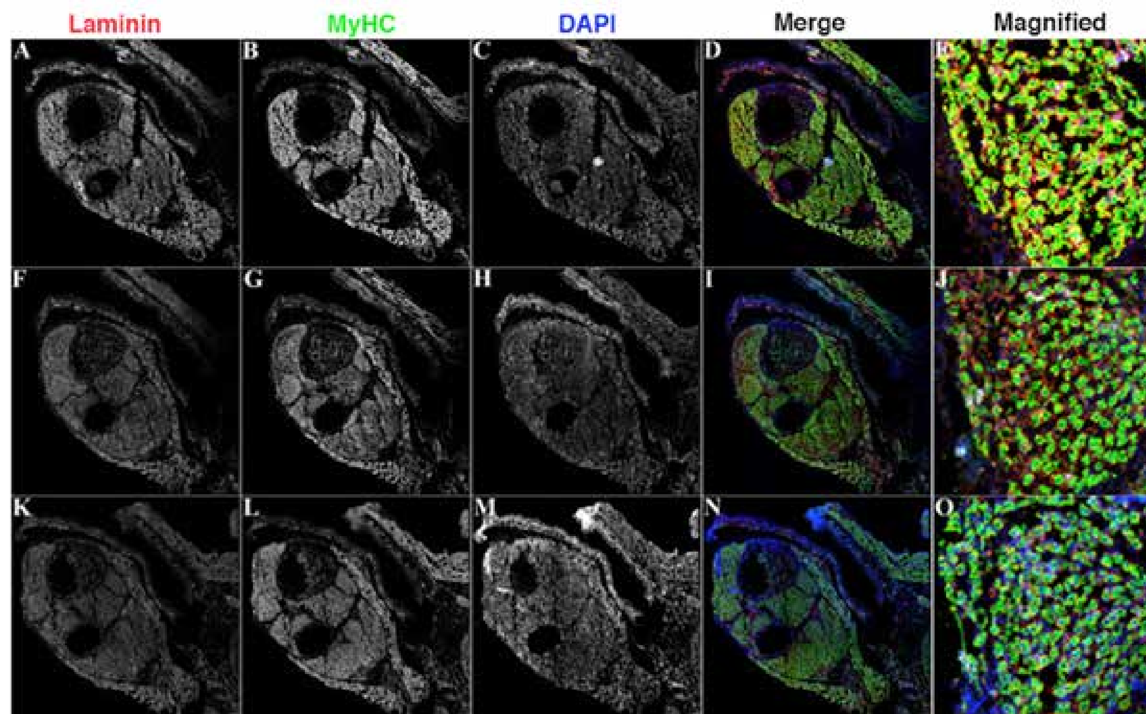

Figure 14: Developmental Myosin Heavy Chain (MyHC) expression in the hind limb at embryonic day 18.5 of mouse development. Confocal maximum projections of adjacent sections through the shank region of the hind limb of an embryonic day 18.5 (E18.5) mouse embryo, stained for Laminin (A, F, K), MyHC-emb (B), MyHC-slow (G), MY32 for MyHC-peri (L), DAPI for nuclei (C, H, M), merge (D, I, N) and magnified views (E, J, O). All MyHCs are expressed in myofibers at this stage.

By E18.5, just prior to birth, all 3 developmental MyHC proteins are robustly detected by immunofluorescence, although by this stage, the antibody used to detect MyHC-peri, MY32, will also start detecting the adult MyHC isoforms (MyHC-IIA, -IIB and -IIX), whose expression initiates around this time (fig. 14). It is also obvious that by this stage, MyHC-slow protein is expressed in fewer myofibers as compared to MyHC-emb and -peri (fig. 2 E, J, O).

The function of one of the developmental MyHC isoforms, MyHC-emb, during embryonic and fetal stages of development has also been characterized. Using a conditional allele for MyHC-emb in mice that we generated, it was found that this MyHC isoform is required to regulate myofiber number, myofiber area and myofiber type during mouse embryonic development. This indicates that MyHC-emb is a crucial regulator of skeletal muscle differentiation and that its absence leads to aberrant skeletal muscle differentiation in vivo.

In order to determine the role of MyHC-emb in myogenic differentiation in vitro, the effect of siRNA mediated MyHC-emb knockdown on C2C12 cell myogenic differentiation was also studied. It was observed that MyHC-emb specific siRNA treatment reduced MyHC-emb transcript and protein levels significantly. Further, knockdown of MyHC-emb resulted in upregulation of MyHC-slow, initial upregulation of MyoD and Myogenin transcription factors followed by their downregulation at the protein level. This suggests

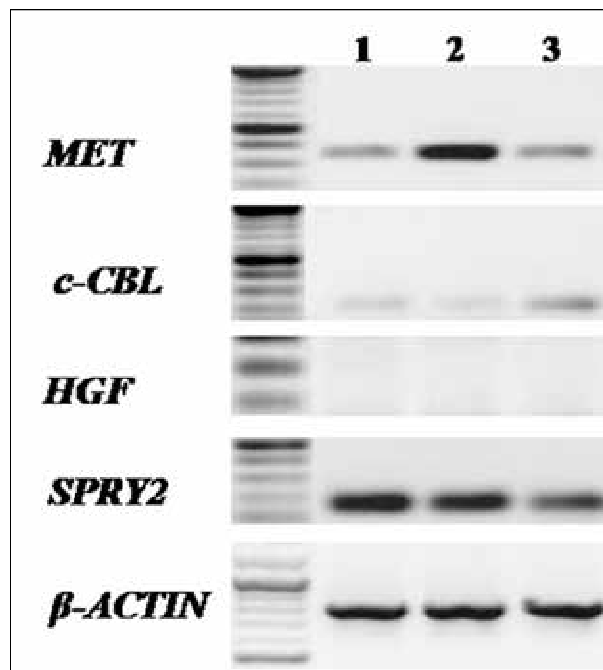

Figure 15: Semi-quantitative RT-PCR for candidate genes on tumor cell lines. Expression analysis for MET, c-CBL, HGF, and SPRY2 with β- ACTIN as control, by semi-quantitative RT-PCR on RMS cell lines CCL-136 an ERMS cell line (1), CRL-2061 an ARMS cell line (2), and A-673 Ewing's sarcoma cell line (3). The RMS cell lines exhibit elevated transcript levels of MET and SPRY2, with reduced expression of c-CBL.

that MyHC-emb is normally required to regulate the rate at which myogenic differentiation occurs. In the absence of MyHC-emb, the undifferentiated stem-like cells underwent rapid differentiation, as shown by the upregulation of MyoD and Myogenin differentiation markers. As further evidence for this, a decrease in the number of reserve cells- the myoblast cells that differentiate to produce myotubes and myofibers, was also observed upon MyHC-embryonic siRNA treatment.

Investigations are also being conducted 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 in RMS has not been clearly understood. RMS cell lines derived from patients, which are mainly of the embryonal (ERMS) or alveolar (ARMS) subtypes, would be used to investigate how Met signaling is regulated in these cancers. It was observed that like MET, levels of a bimodal regulator of receptor tyrosine kinases, SPRY2 are also upregulated in RMS and are investigating whether MET and SPRY2 play any role in regulating each other in RMS (fig. 15).

In the future, efforts to characterize the function of MyHC-emb using the knockout mouse allele that was generated and to gain insights into the role of MyHC-emb in myogenic differentiation in vivo will continue. Genetic crosses will be performed to generate animals with the correct genotype to perform conditional deletion of MyHC-embryonic during development, using Pax3Cre and Pax7iCre driver lines to delete gene expression during embryonic and fetal stages respectively. In addition, the study of in vitro myogenesis by performing siRNA mediated MyHC-embryonic knockdown on C2C12 myogenic cells to understand the role of MyHC-embryonic on in vitro myogenic differentiation will be continued. These studies will be expanded to include other developmental MyHCs as well. Studies on rhabdomyosarcoma (RMS) cell lines derived from patients will also be initiated. to first verify whether c-MET levels are dysregulated in these tumor cell lines. Subsequently, the mechanism behind this dysregulation, specifically focusing on pathways causing ubiquitin-mediated degradation of c-MET, as well as protein interactions that stabilize c-MET will be addressed.

Mechanisms of Cell Division and Cellular Dynamics

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The cells in our bodies are the minimal essential units of life. These cells are highly dynamic entities that proliferate and communicate with each other to enable the complex functions of the body, and malfunctions in which lead to deadly diseases. This program aims to understand, in molecular detail, how cells duplicate and mutually communicate, with a view to gain a fundamental understanding of how cells function. This knowledge could be exploited for future therapeutic intervention against major diseases.

The research program studies the molecular regulation of cellular dynamics. The investigators are examining the molecular mechanisms of cell division and intercellular communication, two vital and highly dynamic cellular processes essential for cell survival, cell proliferation and organism development. The program aims to understand the dynamic molecular regulation of key cellular processes that are important in health and disease. As part of this broad aim, the study aims to understand the molecular mechanisms of mitotic regulation by the intracellular molecular transport motor cytoplasmic dynein. In addition, investigative strategies attempt to uncover the role of the exocytic membrane trafficking machinery during cytokinesis, the physical separation of daughter cells at the end of mitosis. Additionally, the study aims 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. It is hoped that knowledge gained from these studies could be directly exploited towards strategies for the amelioration of human disease conditions.

Major progress was made on one of the projects under this program on novel modes of intercellular communication. The research project has 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 900 nm and often connect cells that are several hundred mm apart. NCs have been implicated in a wide spectrum of crucial cellular functions: 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, communication between 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. This remains a crucial outstanding goal in the field and has been the primary target of investigation in the group over the last few years in this project. Recently, the protein NCTAP was reported essential for NC formation in the literature. While this is the first step towards understanding the molecular regulation of NC biogenesis, there are is very rudimentary knowledge at present on the mechanisms of biogenesis and function of NCs. Moreover, it is unknown which other cellular proteins are involved in NC formation and/ or function. The study aims to elucidate the molecular events controlling NC biogenesis and function by testing the hypothesis 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.

The study had earlier reported the generation of a mammalian cell line stably expressing NCTAP, using which the entire cellular interactome of NCTAP had been determined by mass spectrometry-based interactomic analyses. A shortlisted set of about two dozen proteins that appeared reproducibly as the highest confidence interactors of NCTAP in this cell line, but not in an equivalent cell line generated expressing the empty TAP tag alone were chosen for further exploration. In order to validate the interactions seen by proteomic analyses, multiple strategies were chosen. First, a functional secondary small RNA screen, depleting these high confidence interactors of NCTAP using treatment of the cell line with sequence-specific siRNA combinations ("SMARTpools") was performed. The efficiency of mRNA depletion was ascertained using real time PCR analysis from these cells after 48 hours of treatment with the siRNAs and confirmed to be at least 80% in comparison to negative controls. Cells depleted of these mRNAs were analyzed by high-resolution confocal microscopy and scored for the number of NCs formed per hundred cells as a measure of the ability of these cells to form NC-like structures. A pronounced and reproducible loss of NCs was observed upon depletion of ERC, implicating ERC as a new player in NC formation (fig. 16a). In order to test the converse, ERC was expressed exogenously in the same cells in a suitable mammalian expression vector and observed a marked increase in the number of NCs emanated from cells, thus establishing the functional importance of NCTAP for NC formation.

Verification of the biochemical binding of ERC to NCTAP was performed using affinity precipitation assays followed by immunoblotting. It was confirmed that affinity purification using the TAP tag from lysates of cells stably expressing NCTAP were able to pull down ERC robustly, while lysates from an equivalent empty TAP tag expressing cell line did not show any interaction (fig. 16b). The above results confirmed the specific interaction between NCTAP and ERC and reaffirmed its strong and essential role in NC biogenesis.

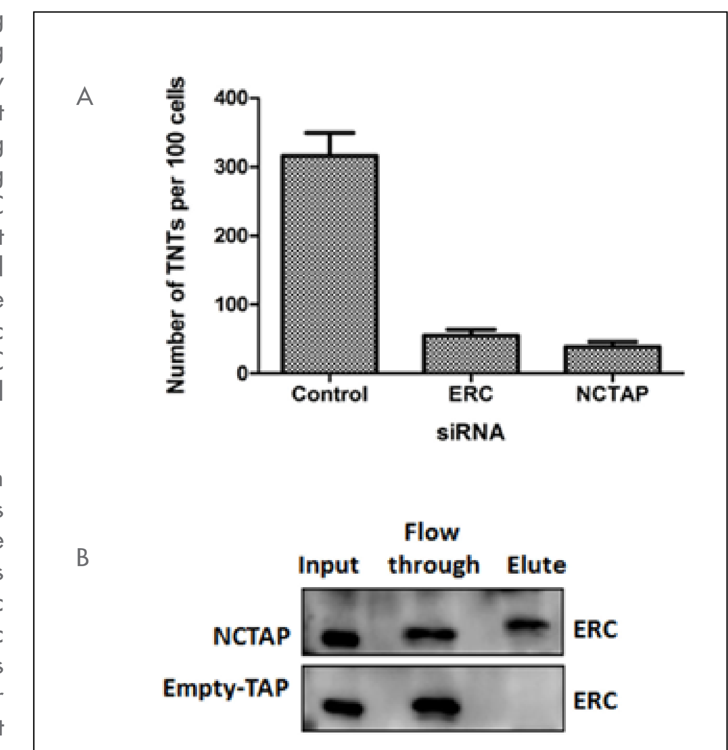

Figure 16: A : The depletion of ERC from cells led to a drastic reduction in their ability to form NCs. B : NCTAP strongly interacts with ERC in immunoprecipitation experiments but not with empty vector controls.

The molecular mechanism through which ERC mediates NC biogenesis was probed. Analysis of the literature revealed that ERC localizes predominantly in the endoplasmic reticulum (ER). Confocal microscopic analysis revealed that NCTAP too is significantly enriched in the perinuclear region in a manner strongly reminiscent of ER staining (fig. 17).

In addition, NCTAP also localizes to the Golgi complex, as determined by colocalization with GM130, a well-known Golgi complex marker. These novel areas of subcellular localization of NCTAP suggested that the protein might interact with ERC in the ER. This hypothesis will be confirmed by ongoing subcellular fractionation assays. It was observed that the depletion of ERC leads to loss of NCTAP from the ER and also from the cell

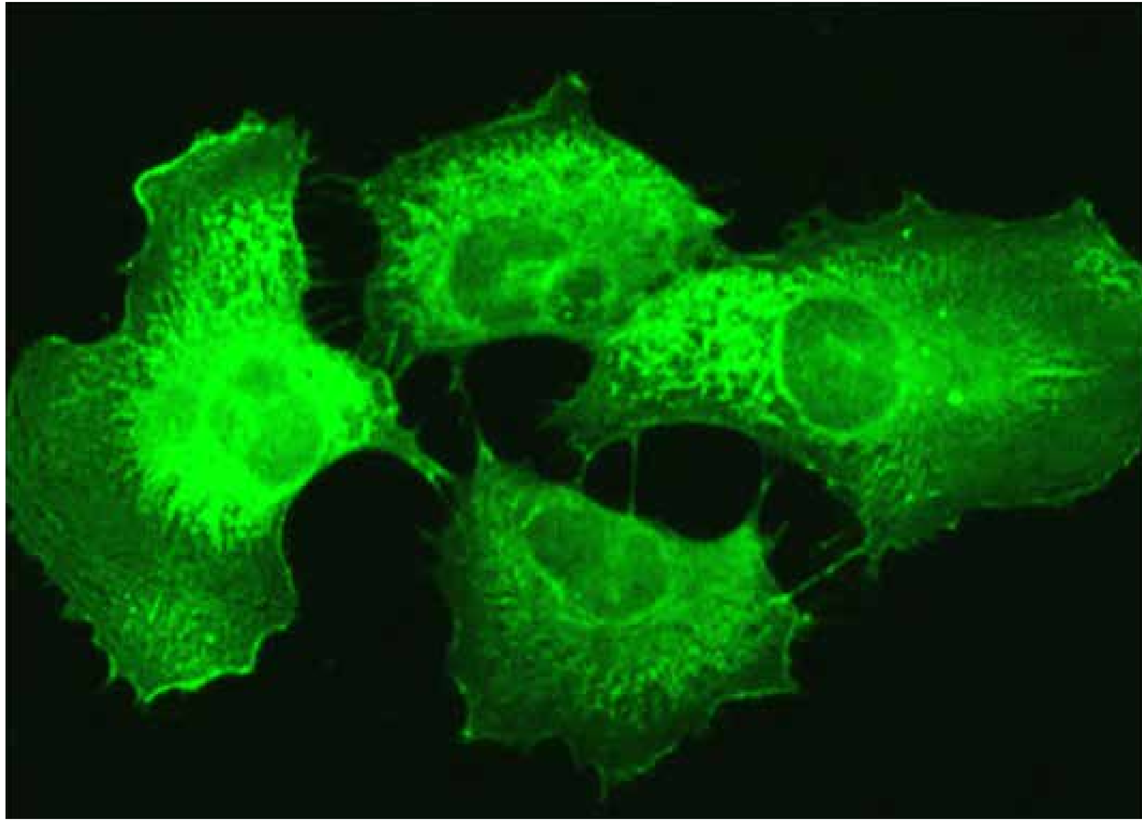

Figure 17: NCTAP (green) expressing stable human osteosarcoma cell line. The image shows the perinuclear enrichment of NCTAP in addition to its known sub cortical localization near the plasma membrane. NCTAP is also present inside the NC like structures (long, thin, strand like structures connecting adjacent cells).

cortex. This result suggests that ERC is required for NCTAP to reach the cortex, which is a prerequisite for NC biogenesis. It was examined whether ERC played a role in NC biogenesis independent of NCTAP, or whether they acted in the same pathway. Strikingly, ERC depletion or ERC overexpression upon depletion of NCTAP by siRNA treatment had no appreciable effect on NC formation, suggesting that ERC acts on NCTAP which subsequently affects NC formation, but does not independently impact NC formation.

Interestingly, ERC depletion did not affect the mRNA levels of NCTAP, but led to a drastic reduction in its protein levels only. The converse was not observed however – depletion of the NCTAP mRNA using sequence specific siRNAs did not impact either the mRNA or protein levels of ERC. These results clearly demonstrated that NCTAP is a downstream target of ERC action. It also confirmed that ERC did not have any transcriptional effect on NCTAP, but only a post-translational one on the protein. The increased expression of ERC using transfection of an exogenous plasmid had the opposite effect, i.e. it stabilized NCTAP protein levels. Such protein stabilization activity is reminiscent of several protein chaperones that help fold and stabilize the translated protein product. Indeed, a survey of the literature confirmed that ERC is a well-known ER based chaperone that helps stabilize other protein substrates in the ER and sometimes up to the Golgi complex and plasma membrane. In order to test whether NCTAP was a substrate of ERC, transgenically expression of various single site mutations of ERC required for its chaperone activity was performed. Expression of these mutants exhibited a dominant negative phenotype that phenocopied ERC depletion in terms of their ability to induce NC formation. It was therefore concluded that the chaperone activity of ERC on its novel substrate NCTAP is essential for the biogenesis of NCs.

Experiments were performed to test the direct binding of ERC to NCTAP using bacterially purified recombinant proteins and glutathione-S–transferase (GST)-tagged affinity purification. The purified recombinant proteins did not bind in affinity purification experiments, nor did recombinantly purified ERC with cellular lysates. These observations pointed to the possibility of either an indirect binding and/or to the requirement of post-translational modifications on either/ both proteins that are important for the binding interaction. Indeed, recombinant NCTAP-GST on glutathione beads bound to cellular lysates containing ERC, suggesting strongly that post-translational modifications on ERC are important for its interaction with NCTAP. Present efforts are focused on determining the minimal determinants on ERC for binding to NCTAP. This knowledge would be vital to identify a minimalistic molecular target on ERC that is important for NC formation through its interaction with NCTAP. Targeting this interaction in the future could provide specific and potent tools to abrogate or modulate NC formation.

The studies in this project are focused on determining the mechanistic bases for formation and function of the important, ubiquitous and intriguing but poorly studied intercellular membrane nanoconduits (NCs). The discoveries reported here have succeeded in unearthing a novel role for an important endoplasmic reticulum chaperone in the biogenesis of NCs. Ongoing efforts are trying to determine whether the interaction between NCTAP and ERC occurs in the ER or in some other cellular compartment(s) using subcellular fractionation assays. Additionally, the path taken by NCTAP from the ER where it appears to significantly enrich, to the cell cortex in the cell where it is also enriched is under investigation. In parallel, efforts are ongoing towards characterization of the NCTAP protein using biochemical and biophysical methodologies, with the objective of obtaining a more complete understanding of the contribution of this protein in NC biogenesis and function. Efforts have also been initiated to understand the mechanistic importance of other high confidence interactors of NCTAP in the biology and function of membrane nanoconduits.

Engineering of Nanomaterials for Biomedical Applications

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This group comprising of researchers with expertise in chemistry and biology are working together on two major aspects of human health, namely cancer and infectious microbial diseases. The goal of their research is to improve the effectiveness of the anticancer drugs and reduce the side effects caused by them either by changing the drug or the drug delivery vehicle. New molecules for treatment of wounds, tuberculosis, and catheter-induced and urinary tract infections are also being synthesized.

An interdisciplinary approach exploiting synthetic chemistry, cell biology, microbiology, cancer biology and nanotechnology is being employed to address the challenges in the area of cancer biology and infectious diseases and to develop nanomaterials for effective therapeutics for cancer and infectious diseases.

Efficient liposomal-based drug delivery systems using different kinds of phospholipids have been commercialized for treatment of cancer and fungal infections. The encapsulation of active drug ingredients either in the aqueous or lipid bilayer compartments form the premise of the strategy for liposomal formulations, where drug entrapment efficacy and their release kinetics is contingent on the chemical nature and composition of lipid ingredients. Low drug encapsulation, poor retention efficiencies, instability of the liposomes and their uptake by the reticulo-endothelial system, non-specific leakage of the encapsulated drug and poor accumulation at tumor sites are major limitations of the encapsulation strategy used for anticancer drug delivery. Therefore, designing of phospholipids for drug delivery is an active area of research; and recent studies have stressed on fine-tuning of the physicochemical, mechanical and structural properties of the lipids to control the release rates of encapsulated drugs.

Design and drug encapsulation efficacy

Three bile acids — lithocholic acid (LCA), deoxycholic acid (DCA), and cholic acid (CA)—were selected and three bile acid-phosphocholine (BPC)-derived phospholipids designed, where the phosphocholine head group was appended to the 3′-hydroxyl group of bile acids (fig. 18). Next, a mixture of BPC, EggPC and DSPE-PEG 2000-amine in a weight ratio of 1:1:0.2 we used to form liposomes, where the Egg PC and DSPE-PEG 2000-amine promoted efficient encapsulation and stealthing of liposomes respectively. Quantification of phospholipid content analysis showed on average 73%, 47%, and 44% retention of phospholipids respectively after extrusion in case of LCA-PC, DCA-PC and CA-PC liposomes. Dynamic light scattering (DLS) studies showed a monomodal size distribution of 100-110 nm with a polydispersity index of 0.1-0.2 and zeta potential ranging from 12-22 mV.

The doxorubicin (a model water-soluble anticancer drug) encapsulation ability of BPC liposomes was tested using a remote drug loading method, and ~58% drug encapsulation for LCA-PC and ~75% drug encapsulation for both DCA-PC and CA-PC liposomes was observed. The presence of free hydroxyl groups increased the fluidity of DCA-PC and CA-PC liposomes, which allowed them to entrap additional doxorubicin molecules in the lipid bilayer than the non-hydroxylated LCA-PC liposomes. Estimation of the phospholipids retained by liposomes revealed that LCA-PC liposomes had higher lipid content (73%) than DCA-PC and CA-PC liposomes (~40%) because of better packing of LCA-PC in the membranes. Therefore, the entrapment efficiency of DCA-PC and CA-PC liposomes was around 38%, whereas it was ~18% for LCA-PC liposomes.

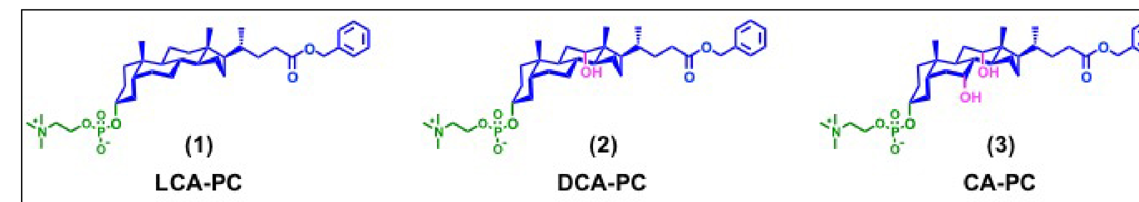

Figure 18: Molecular structures and schematic presentation of Lithocholic Acid (LCA), Deoxycholic Acid (DCA) and Cholic Acid (CA) derived phospholipids LCA-PC, DCA-PC and CA-PC.

Drug release kinetics

The release kinetics of doxorubicin from BPC liposomes were investigated under the following conditions: (a) physiological pH using phosphate buffer saline (PBS, pH 7.4), (b) acidic tumor microenvironment (acetate buffer, pH 5.0), and (c) blood serum [10% fetal bovine serum (FBS) in PBS, pH 7.4] at 37°C. The initial burst release of doxorubicin was observed from all the three liposomes at pH 7.4, and the cumulative doxorubicin release was below 50%. Although a slight increased rate of doxorubicin release at both pH 7.4 and pH 5.0 was observed, there were no distinct differences in drug release among the three liposomes. Marked differences in the rates of doxorubicin release in FBS were observed among these liposomes as serum proteins are known to interact with liposomes and cause burst release of the encapsulated contents. Faster release rate and enhanced doxorubicin release content (~80%) from the hydroxylated DCA-PC and CA-PC liposomes were observed, whereas the LCA-PC liposomes maintained a sustained release of doxorubicin with a maximum ~60% release of contents. The differential drug release patterns of BPC liposomes might arise from the distinct packing of phospholipids in the lipidated liposomes, which allows faster release of the loosely encapsulated doxorubicin from DCA-PC and CA-PC liposomes. Therefore, the effect of BPCs on membrane fluidity using diphenylhexatriene (DPH)-based membrane probe was investigated.

Membrane rigidity studies

The anisotropy of DPH, a rigid fluorophore, is reduced when it transits from a tightly packed environment to fluidic conditions as perturbations in the internal packing of membranes allow random movement of DPH. Anisotropy studies revealed marked differences in the rigidity of hydroxylated and non-hydroxylated phospholipid liposomes. Membranes containing LCA-PC were highly rigid due to better packing efficiency of LCA-PC lipids in membranes, whereas membranes containing DCA-PC and CA-PC were comparatively fluid in nature. Rigid packing of LCA-PC causes slow release of doxorubicin, whereas the fluidic nature of hydroxylated phospholipids (DCA-PC and CA-PC) induces enhanced encapsulation with faster drug release kinetics. Therefore, distinct differences in membrane rigidity of lipidated liposomes that vary in number of free hydroxyl groups are responsible for marked differences in encapsulation and drug release from BPC liposomes.

In vitro cytotoxicity studies

Anticancer activities of doxorubicin-encapsulated BPC liposomes were then studied in murine (4T1) and human (MDA-MB-231) breast cancer cell lines. Interestingly, it was observed that human MDA-MB-231 cells were less susceptible to liposomal formulation as compared to murine 4T1 breast cancer cells. As expected, a 48-hour exposure was more toxic to the cells as compared to a 24-hour exposure. After 24 hours of exposure to 4T1 murine cancer cells, drug entrapped CA-PC liposomes were more toxic as compared to drug encapsulated LCA-PC liposomal formulations due to fast drug release from drug-entrapped CA-PC liposomes. All drug-entrapped BPC liposomal formulations in general were found to induce cell death in both the cell lines after 48 hours, indicating effective release of doxorubicin from the liposomes.

In vivo antitumor activities

The frequency of chemotherapy schedules depends on the elicited toxicity, tumor regression, and survival response of the patients. Frequent chemotherapeutic schedules enhance tumor regression, albeit with high toxicity and poor survival. Therefore, to understand the impact of BPC liposomes on anticancer activity, toxicity, and survival, the potency of doxorubicin encapsulated BPC liposomes against 4T1 murine breast cancer model

in BALB/c mice was evaluated. A 40-50% reduction in tumor volume was observed upon treatment with doxorubicin loaded BPC liposomes as compared to untreated control whereas Adrisome™ treated mice did not show any progression of tumor growth (fig. 2A). Drug release from the liposomal formulations impacts tumor regression, toxicity, and survival of the mice as tumor regression with enhanced toxicity or poor survival is not recommended. The survivability of tumor-bearing mice on different treatments was therefore compared. Treatment with doxorubicin alone without any carrier could not enhance the survivability of mice due to its toxic nature. A differential impact on mice survival on treatment with drug-encapsulated liposomes was observed irrespective of the differences in body weights of the drug-treated mice (fig. 2B). Mice treated with LCA-PC liposomes showed significant improvement in survival than that observed with DCA-PC and CA-PC liposomes (fig. 2C), whereas mice treated with drug-loaded CA-PC liposomes died before the untreated tumor-bearing mice in spite of 50% tumor regression due to the high toxicity of the free drug present in the blood circulation. A similar effect was observed during in vitro studies where doxorubicin-entrapped CA-PC liposomes are more toxic to murine cancer cells as compared to drug-entrapped LCA-PC liposomes. Therefore, rigid LCA-PC liposomes triggered a significant increase in mice survival compared to the poor survival rate of the DCA-PC- and CA-PC-liposomes treated mice. The un-entrapped doxorubicin treated mice showed sudden weight loss and decline in survival.

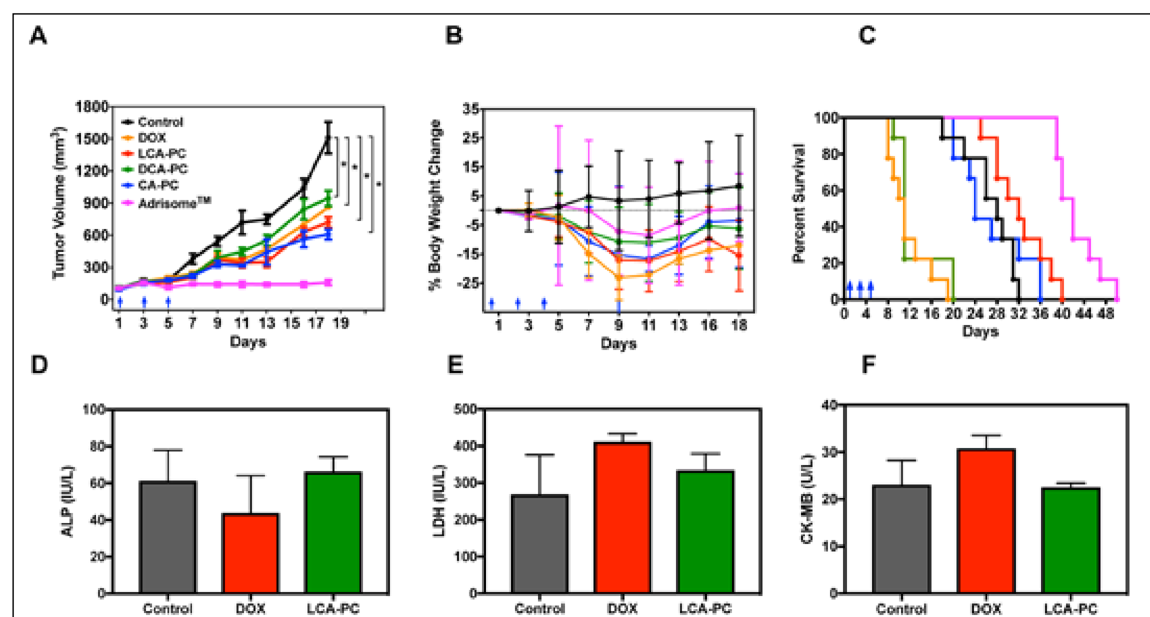

Figure 19: (A) Change in tumor volume, (B) body weight, and (C) survival of 4T1 tumor bearing Balb/c mice treated with doxorubicin and doxorubicin entrapped BPC liposomes; (D-F) Hepatic alkaline phosphatase (ALP) (D); skeletal muscle lactate dehydrogenase (LDH) (E); and cardiac muscle specific toxicity marker (CK-MB) (F) toxicity markers post 24h of treatment with doxorubicin and doxorubicin encapsulated LCA-PC liposomes in 4T1 tumor bearing mice.

To investigate this, the levels of lactate dehydrogenase (LDH; muscle tissue marker), cardiac muscle-specific creatinine phosphokinase (CK-MB), and alkaline phosphatase (ALP for liver toxicity) were estimated and compared in mice serum after 24 hours of treatment with free doxorubicin and doxorubicin-entrapped LCA-PC liposomes (fig. 19D-19F). A significant decrease in ALP levels was observed; and an increase in circulatory levels of LDH and CK-MB upon doxorubicin treatment, whereas the doxorubicin-entrapped LCA-PC liposomes did not elicit any toxic response in the tumor-bearing mice. Higher circulatory levels of these markers in mice treated with free doxorubicin could possibly explain the cardiac toxicity and loss in muscle mass of these animals.

Pharmacokinetics and bio-distribution studies

Pharmacokinetic studies revealed ~1.5-fold increase in the plasma concentration of doxorubicin on treatment with drug entrapped LCA-PC liposomes as compared to doxorubicin alone with similar mean resident time (MRT) for LCA-PC liposomes and doxorubicin. There was ~1.5-fold increase in drug availability using doxorubicin entrapped liposomes as compared to doxorubicin alone. Bio-distribution studies revealed a three-fold increase in doxorubicin concentration at the tumor site upon treatment with LCA-PC liposomes than that observed with doxorubicin alone (fig. 20A-20C). Negligible doxorubicin concentration was observed in vital organs such as heart and lungs. Treatment with doxorubicin-entrapped LCA-PC liposomes lowered the accumulation of doxorubicin in the kidneys, implying enhanced blood circulation compared to that observed upon treatment with free doxorubicin.

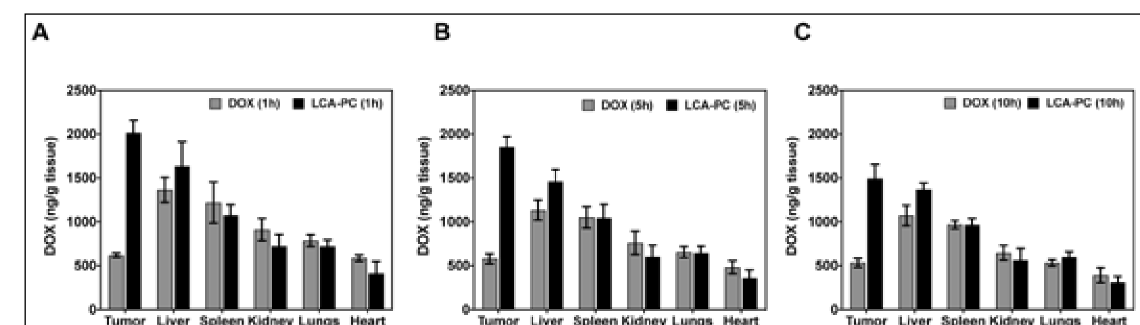

Figure 20: Bio-distribution of doxorubicin and doxorubicin entrapped LCA-PC liposomes at different time points 1hour (A), 5 hours (B), and 10 hours (C) in 4T1 tumor bearing Balb/c mice.

In the future, chimeric nanoparticles containing a combination of anticancer and anti-angiogenic drugs encapsulated in a single nanoparticle will be engineered. The suitable chemical linkages and encapsulation strategies will allow the sequential release of these drugs in a desired manner. These chimeric nanoparticles will help in spatio-temporal targeting of tumor progression by targeting angiogenesis and cell proliferation; and help in getting enhanced tumor regression with increased survival. As chemotherapy regimens often lead to emergence of drug resistance in cancer cells, lipidomic and genomic approaches will be used to uncover the effect of the engineered drug delivery systems on the emergence of drug resistance. This would help in developing combination therapy regimens for combating drug resistance.

Dyneins and Development: Role of Dynein Light Intermediate Chains in Embryonic Divisions and Vertebrate Embryogenesis

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This program aims to study the role of cell division (mitosis) in regulating the proper development of an embryo. Correct cell division is vital for crucial developmental events such as tissue and organ formation and is therefore tightly regulated in the embryo. Aberrant embryonic cell divisions result in abnormal tissue architecture leading to embryonic death and/or disorders such as microcephaly and Down's syndrome. The study uses zebrafish and human cells as experimental laboratory model systems to understand the importance of key embryonic proteins in ensuring proper early embryonic cell divisions. Knowledge gained from the work will advance the understanding of early developmental events in vertebrates and could be exploited to design therapies against developmental disorders.

Mitosis in the embryo is a tightly regulated process, involving a variety of cytosolic proteins in a complex signaling network. Interestingly, developmental cues from the embryo also impart instructive signals to regulate mitotic events such as spindle orientation and spindle positioning. The objective of this program is to understand the molecular crosstalk between mitotic events and developmental signals in the developing embryo. To this end, the aim is to study the role of cell division in governing vertebrate embryogenesis. In particular, the focus is on understanding the function of the intracellular microtubule based motor dynein in regulating mitosis during embryonic development. Further, the aim is to decipher the molecular crosstalk of dynein with extracellular developmental signals in shaping the embryogenic program.

Cell division forms the underlying basis for embryonic development. Mitotic aberrations result in cell fate misspecification, abnormal tissue architecture and aneuploidy in the developing embryo. Thus, it is imperative to study the molecular mechanisms regulating cell divisions during development. This study aims to unearth important mechanistic contributions of dynein in ensuring proper mitotic divisions in the vertebrate embryo. The study will test the hypothesis that dynein performs crucial functions in regulating the fidelity of early embryonic divisions, in a manner akin to its roles demonstrated in cell culture systems. Regulation of these early divisions by dynein would shape proper development of the embryo and the depletion of or defects in dynein would thus lead to abnormal cell division and consequently cause developmental defects. The zebrafish embryo and mammalian cell lines are employed as model systems to answer these questions, since mammalian dynein subunits are evolutionarily conserved in the zebrafish.

The Role of the Dynein Motor in Regulating Early Vertebrate Development

A vital aspect of dynein biology that is under-explored is its role in embryonic development. Cytoplasmic dynein is a minus-end directed microtubule motor protein complex that plays key roles in mitotic progression. Cytoplasmic dynein consists of many subunits - Heavy Chains (HCs), Intermediate Chains (ICs), Light Intermediate Chains (LICs), Light Chains (LCs) and other cargo binding proteins such as Roadblock and Tctex-1. Cytoplasmic dynein 1 (henceforth called dynein) is involved in multiple mitotic functions like spindle formation and organization, spindle positioning, chromosome movement and alignment at the equatorial plate at metaphase and spindle assembly checkpoint (SAC) silencing prior to anaphase onset. Dyneins regulate spindle positioning, which is crucial to form daughter cells of equal size. A "cortical pulling mechanism" involving cytoplasmic dynein is implicated in determining spindle positioning. Dynein molecules are attached to the cell cortex and exert pulling forces on the plus ends of the astral microtubules. Recently, dynein Light Intermediate Chains (LICs) have been shown to play important roles in silencing the SAC by transporting checkpoint proteins from attached kinetochores towards the spindle poles. Further, knockdown experiments suggest that LIC2 is required for centrosomal positioning. Recent studies show that depletion of LIC2 by a siRNA mediated approach results in several metaphase defects in HeLa cells. LIC2 plays an important role in inactivation of the SAC by removal of SAC proteins during metaphase to anaphase transition. In LIC2 depleted cells, the SAC proteins fail to detach from the kinetochores, resulting in prolonged metaphase and failure to advance into anaphase. Further, it has been shown that LIC2 regulates spindle positioning and orientation during metaphase, very likely by ensuring astral microtubule nucleation and their proper anchoring at the cell cortex. LICs therefore play important roles in cell division.

During embryonic development, proper spindle orientation in blastomeres is required for distribution of cell fate determinants and fate specification. The aim of this study is to understand the functional contribution of the dynein LIC subunits during mitosis in the formation of the bipolar spindle and in establishing proper spindle orientation in developing zebrafish embryos. The hypothesis is that zebrafish LICs are required for these functions and that loss of or defects in these subunits impair proper spindle formation and orientation, leading to developmental defects. This study therefore aims to determine the functions and mechanistic role(s) of the dynein LIC subunits during vertebrate embryonic development. The cytological phenotypes in the blastomeres during early divisions upon LIC depletion are being characterized. The effect of LIC depletion on morphogenetic events leading to defects at later stages of development will be assessed and attempts made to discern key molecular mechanisms of action of the LICs using a combination of embryology and cell culture studies. In addition, the roles and molecular mechanisms of key LIC interactors in achieving mitotic functions would be probed.

In order to determine the functions of the LICs in the embryonic development of zebrafish, gene knockdown approach by using sequence specific morpholinos was used to deplete the LICs and the resultant phenotypes characterized by confocal microscopy (fig. 21, 22). Morphant embryos showed increased mitotic index at early stages of development (fig. 21). The LIC morphants also exhibit multiple mitotic defects such as increased spindle length and chromosome congression defects (fig. 22). Such mitotic aberrations in early development may result in unequal distribution of cell fate determinants and altered cell fate in the resulting daughter cells.

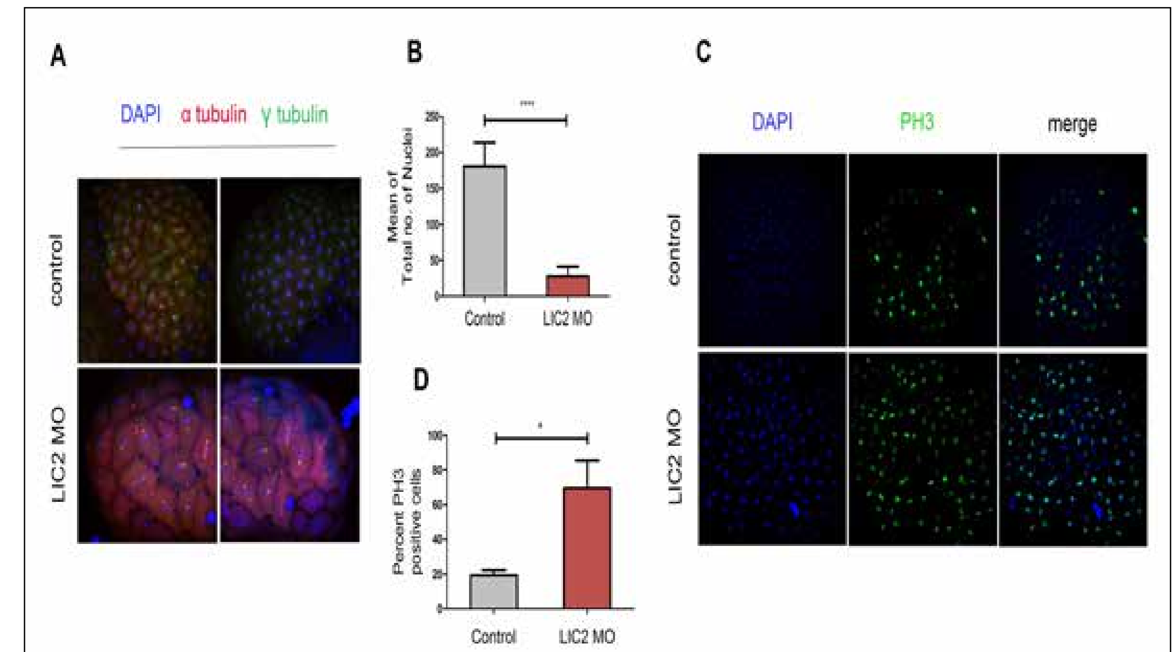

Figure 21: Zebrafish LIC2 depletion leads to mitotic defects in early embryonic divisions. (A) Cytology of blastomeres upon LIC2 depletion. Each representative image shows the top surface view of the embryo at 3.3 hpf, recreated from confocal z-stacks. Control = embryos injected with standard control MO. Embryos are stained for microtubules (red), centrosomes (green) and chromosomes (blue). (B) Total average number of nuclei per embryo in surface blastomeres of time-matched embryos (minimum 3 experiments, n = 10 control and 23 LIC2 depleted embryos). (C) Sum projections of confocal z stacks showing mitotic cells labelled with phosphohistone 3 (PH3, green) and chromatin (DAPI, blue). (D) Fraction of PH3 positive cells upon respective treatment, n = 23 and 15 embryos respectively for uninjected and LIC2 MO treatment from 2 experiments.

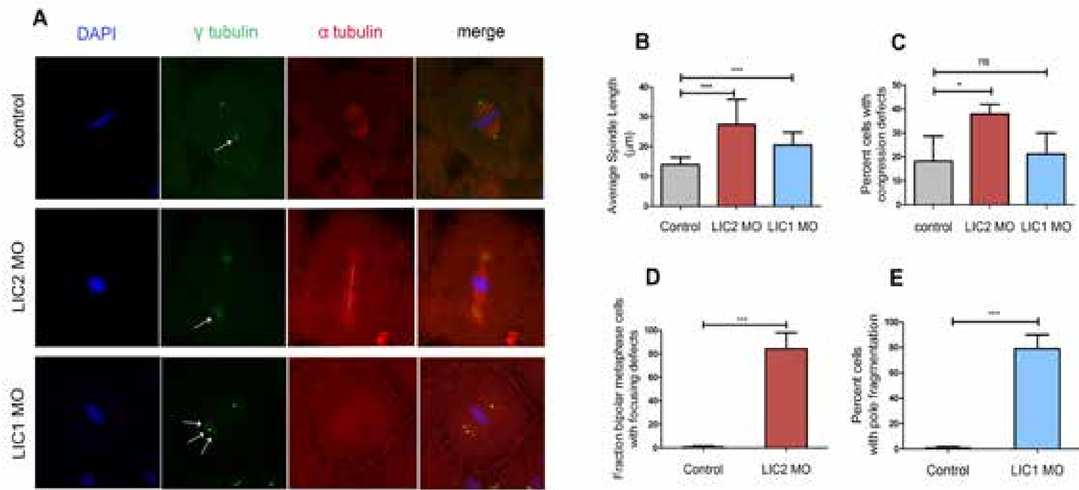

Figure 22: Zebrafish LIC2 depletion leads to mitotic defects in early embryonic divisions. A) Confocal images of surface blastomeres showing longer mitotic spindles (white dotted line), unfocused spindle poles (white arrow) and chromosome congression defects in zLIC 1 and 2 morphants. Chromosomes (DAPI, blue), spindle poles (-tubulin, green) and microtubules (-tubulin, red) are immunostained as indicated. B) Average spindle length in surface blastomeres. n = 139 control cells (30 embryos), 118 metaphase cells for LIC2 depletion (26 embryos) and 52 metaphase cells (10 embryos) for LIC1 depletion, across a minimum 3 experiments each. C) Fraction of blastomeres showing chromosome congression defects from the embryos in F. D) Fraction of blastomeres showing spindle pole focusing defects upon LIC2 depletion. n = 69control metaphase cells (12 embryos) and 95 metaphase cells (16 embryos) across a minimum of 3 experiments each. E) Fraction of blastomeres showing spindle pole fragmentation upon LIC1 depletion. n = 52 metaphase cells (10 embryos) across 3 experiments. Error bars are mean +/- SD.

Molecular crosstalk of dynein with extracellular developmental signals in shaping the embryogenic program

Preliminary investigations reveal interesting developmental defects in the dynein morphants at later stages of development. Based on these phenotypes, it is hypothesized that extracellular developmental cues such as Wnt signaling members interact with dynein to regulate mitotic functions in the actively dividing blastomeres (fig. 23). Recent studies showed that key downstream effector of the canonical Wnt pathway are required for centrosome separation and maintenance of spindle bipolarity. Further, dyneins co-localize with Wnt effectors at the cell-cell adherence junctions. In a mitotic cell, dynein and Wnt effectors such as -catenin both localize to the centrosome and the cortical regions, suggesting that they may interact to perform mitotic functions such as spindle orientation. Indeed, the interphase interaction of dynein with -catenin is known to occur at adherence junctions.

Keeping these studies in mind, downstream effectors of proliferation-inducing extracellular cues such as Wnt signaling are expected to interact with dynein in the cell to regulate spindle orientation (fig. 23). The mitotic spindle could be oriented by a combination of three possible pathways. The first pathway regulating orientation

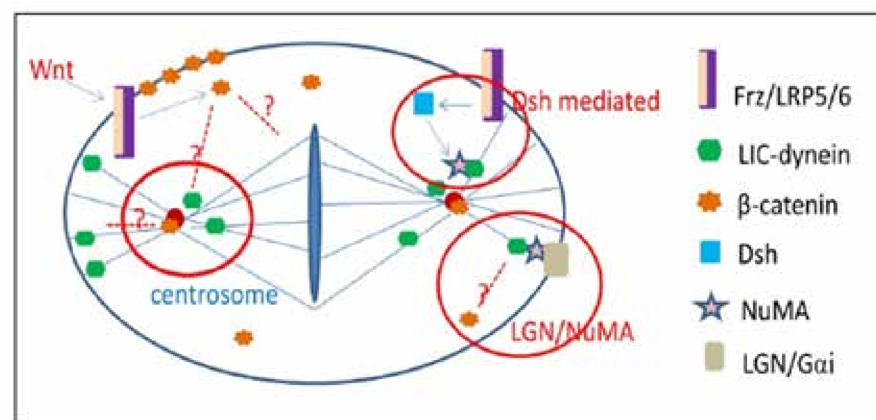

Figure 23: Schematic representation of various pathways involved in spindle orientation. Orientation of the mitotic spindle maybe regulated at the centrosome, at the cell cortex involving LGN-NuMA pathway or the Dishevelled (Dsh)-NuMA pathway.

involves the dynein at the centrosome. The second pathway involves LGN-NuMA-dynein complex interaction at the cortex to regulate spindle orientation. Finally, orientation may be regulated by the non-canonical Wnt pathway involving Dishevelled (Dsh) – NuMA-dynein. Strategies have been initiated to delineate the importance of the interaction between dynein and Wnt pathway effectors using both cell culture and zebrafish embryos as model systems. In addition, several potentially novel interactors of the dynein motor may play key roles in dynein function during mitosis based on recent unpublished data. The project aims to understand the role of one or more of these candidates and their interactions with dynein in mitosis and in embryonic development.

In this study, the functions and mechanistic role of key dynein subunits during vertebrate embryonic development would be determined. Dynein-depleted embryos using morpholino based gene knockdown approach would be characterized for gross morphological defects at various developmental stages. These morphants will be analyzed for mitotic defects at the cellular level, which may form the basis for gross morphological phenotypes. In the second part of the study, the molecular mechanism by which dynein mediates various mitotic events in the actively dividing embryo would be probed. It is hypothesized that extracellular developmental cues from different regions of the embryo play a key role in regulating dynein mediated mitotic functions. Indeed, it was observed that when LIC2-dynein subunit is depleted, defects in dynein dependent mitotic events such as regulation of spindle length, focusing of the spindle poles and chromosome congression appear during early development.

Molecular Intricacies that Regulate and Execute Effector-Triggered Innate Immunity in Plants

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Plants under the constant risk of pathogen attacks have evolved a sophisticated defense system to combat the threats. This intricate immune network present in each cell is conditioned to balance energy requirements of a defense response through a proportional toning down of processes that regulate growth and developmental functions. At the molecular level this involves recruiting proteins that serve the dual function of associating with defense mediators and in parallel coordinating via signaling messengers with cellular homeostasis processes. These signaling routes are being explored with the broad aim to improve disease resistance in economically important crops.

Defense responses in plants involve intricate signal transduction networks that impose limitations on processes that promote regular growth and development. This research program traces the molecular mechanisms of immune trigger, signaling routes and its execution with a broad aim to biotechnologically improve the plant combat capabilities without associated fitness or yield costs.

Intricate signaling routes, originating from 'hub proteins' in association with central defense modulators, impart a spider web-like architecture to immune networking in plants. Upon an immune elicitation, this structured organization facilitates balancing of energy requirements required for defense responses through transitory modulations of other general homeostatic processes. Pathogenic effectors cause perturbations of specific protein-protein interactions within an immune complex, which probably generates the immune trigger. However, the mechanism of translation of such disturbances to downstream signaling routes thus causing massive transcriptomic changes remains unknown. This study investigates the role of inositol phosphates (InsPs) and lipid-conjugated InsPs-the phosphatidylinositols (PtdIns) as signaling mediators of plant defenses. The research focuses on characterizing the strategic deployment of immune complexes on PtdIns-related interfaces and their mode of modulation by pathogenic effectors as well as on deciphering defense signaling routes mediated by InsPs. In addition, the study aims to elucidate defense response-imposed impingement on regular developmental processes and their crosstalk.

InsPs are versatile signaling messengers owing to their highly polar nature and to their potency to covalently attach multiple phosphate groups. However, unlike animal systems, InsPs in plants have been mainly considered as cellular stores for phosphate. Also known as phytate, $InsP_6$ (inositol hexakisphosphate) is the most abundant phosphate-containing compound in seeds, accounting for an enriched energy reserve harnessed during germination. Ruminants fed on seed-based diets are unable to hydrolyze $InsP_6$, which upon its release chelates essential metal ions such as iron, zinc and calcium present in soil, making them unavailable to plants. To reduce this anti-nutrient property, extensive biotechnological efforts have focused on generating low phytic acid (lpa) seeds through modulation of InsP biosynthesis pathways. However, the recent identification of several InsPs as signaling messengers especially as co-factors in hormonal pathways has raised serious concerns on negative side effects on growth and yield of engineered lpa plants. Further on, studies in this program have also implicated specific InsPs at the forefront as signaling messengers of immunity, thus suggesting a careful evaluation of 'combat capabilities' of lpa plants.

To gain better insights, this study has undertaken a comprehensive investigation into the roles of specific InsPs in defense responses. The model plant system Arabidopsis thaliana and the devastating hemi-biotroph Pseudomonas syringae pv tomato (strain DC3000) pathosystem have been utilized for the investigations. Individual approaches undertaken in the studies are described below.

Defensive capabilities of lpa and other InsP biosynthesis and metabolism mutants

T-DNA insertional mutant lines of key InsP biosynthesis and metabolism genes were obtained. These mutants represent gene knockouts/knockdowns that cause accumulation of specific InsPs allowing them to be tested for their role in defense. While some of the mutants tested have been previously designated as bona fide lpa mutants, the others remain uncharacterized. Table 1 summarizes the analysis so far:

S. No.	Mutant name	Gene disrupted	Reaction catalyzed	lpa mutant	Susceptibility to DC3000 (compared to wild-type)
1.	ipk1-1	INOSITOL PENTAKISPHOSPHATE KINASE 1 (IPK1)	Converts $InsP_5$ to $InsP_6$	Yes	Enhanced resistant
2.	ipk2 -1	INOSITOL (1,4,5) P_3 3/6-KINASE 2 (IPK2)	Converts $InsP_3$ to $InsP_4$ and $InsP_5$	No	As wild-type
3.	ipk2b-1	INOSITOL (1,4,5) P_3 3/6-KINASE 2b (IPK2b)	Converts $InsP_3$ to $InsP_4$ and $InsP_5$	Yes	As wild-type
4.	mik-1	MYO-INOSITOL KINASE 1 (MIK1)	Converts myo-inositol to myo-inositol 3-phosphate	Yes	As wild-type
5.	itpk1-1	INOSITOL (1,3,4) P_3 5/6-KINASE (ITPK1)	Converts $InsP_3$ to $InsP_4$ and $InsP_5$	Yes	Enhanced resistant
6.	itpk3-1	INOSITOL (1,3,4) P_3 5/6-KINASE (ITPK3)	Converts $InsP_3$ to $InsP_4$ and $InsP_5$	No	As wild-type
7.	itpk4-1	INOSITOL (1,3,4) P_3 5/6-KINASE (ITPK4)	Converts $InsP_3$ to $InsP_4$ and $InsP_5$	Yes	Homozygous mutant lethal

Table 1: Summary of pathogenicity assays on InsP biosynthesis and metabolism mutants. T-DNA mutagenized plants were tested for disease susceptibility in quantitative growth curve assays using the DC3000 strain.

The above results clearly demonstrate differences in combat capabilities for the lpa mutants. Interestingly, the enhanced resistance observed for ipk1-1 and itpk1-1 mutants also manifests in phenotypic growth defects that reduces seed set in the mutant plants. Thus, a favorable lpa feature has a negative consequence on yield and therefore should be seriously considered when implemented on crop plants. Combinatorial crosses of the above lpa mutants amongst themselves and with plants harboring mutations in known central defense regulators would be generated to decipher the signaling routes of different InsPs.

Differential partitioning of defense-related transcripts in ipk1-1

$InsP_6$ is a key cofactor for the nuclear pore complex (NPC) protein LOW EXPRESSION OF OSMOTICALLY RESPONSIVE GENES 4 (LOS4), mediating nucleocytoplasmic export of mRNAs. The ATPase activity of LOS4 is stimulated in the presence of $InsP_6$. Although ipk1-1 plants display mRNA export defects, elevated proteins of positive defense regulators suggest that partitioning of the cognate transcripts are not affected by the export deficiencies. RNAseq data of cytoplasmic and nuclear pool of mRNA from both wild-type and ipk1-1 plants were obtained. A summary of transcriptomic changes is described in Table 2 below.

Sample pairs	Down-regulated	Up-regulated	Baseline
ipk1-1 Cytoplasm versus Wild-type Cytoplasm	74	98	22476
ipk1-1 Nucleus versus Wild-type Nucleus	139	177	19592

Table 2: Comparative changes in cytoplasm and nuclear localized transcripts in ipk1-1 versus the wild-type plants. Total mRNA isolated from the indicated compartments were sequenced and analyzed at Bionivid Inc.

Initial data analysis identified selective cytoplasmic partitioning of transcripts coding for positive defense regulators. Although transcripts of previously characterized negative regulators reduced, they surprisingly did not demonstrate any alteration in nucleocytoplasmic partitioning. Additionally, partitioning of housekeeping genes also remained unaffected in the ipk1-1 plants. Selected candidates from the RNAseq data were validated and a similar profile detected via qPCRs on nuclear and cytoplasmic RNA pools in the wild-type and ipk1-1 plants. The data introduces selective import of defense-related transcripts as a function of the plant immune system.

It was demonstrated that both IPK1 and ITPK1 genetically function as negative defense regulators. Ipk1-1 itpk1-1 double mutants are being generated to investigate molecular link, if any, between these proteins. In addition, each individual mutant is also being genetically crossed to plants deficient in immunity. For example, eds1-2 and sid2-1 plants are deficient in salicylic acid (SA) mode of immune signaling and corresponding double mutants would determine whether IPK1 and ITPK1 suppress SA as a mode of defense repression.

Similar to ipk1-1, a null-mutant of a nucleoporin MODIFIER OF SNC1-3 (MOS3) was also defective in nucleocytoplasmic mRNA partitioning. However, unlike ipk1-1, a mos3-3 mutant was more susceptible to P. syringae suggesting that mRNA export regulations influence immune outcomes. Ipk1-1 mos3-3 double mutant plants are being generated to address a possible crosstalk between these proteins and to elucidate whether defense alterations in an individual mutant parent are restored in the double mutant progeny. Subsequently, a nucleus versus cytoplasmic RNAseq would also be performed on these plants and compared to the existing data on ipk1-1 to identify transcripts that are selectively partitioned during a defense response.

Components of an immune complex demonstrate overlap in interactions with specific membrane lipids

The key negative regulator SUPRESSOR OF RPS4-RLD 1 (SRFR1) forms multimeric membranous associations with the positive defense-mediator ENHANCED DISEASE SUSCEPTBILITY 1 (EDS1) and specific R proteins in a 'resistasome' complex and prevents its mis-primed activation. In order to trace the membrane locales of these associations, in vitro lipid-binding assays were performed on lipid-coated strips. Towards this, EDS1 was expressed and purified from E. coli extracts. In the lipid-binding assays, EDS1 demonstrated specific binding to Phosphatidic acid (PA) and Phosphatidylserine (PS) (fig. 24). However, purified GFP, which was used as a negative control, did not show binding to any of the lipids present on the strip.

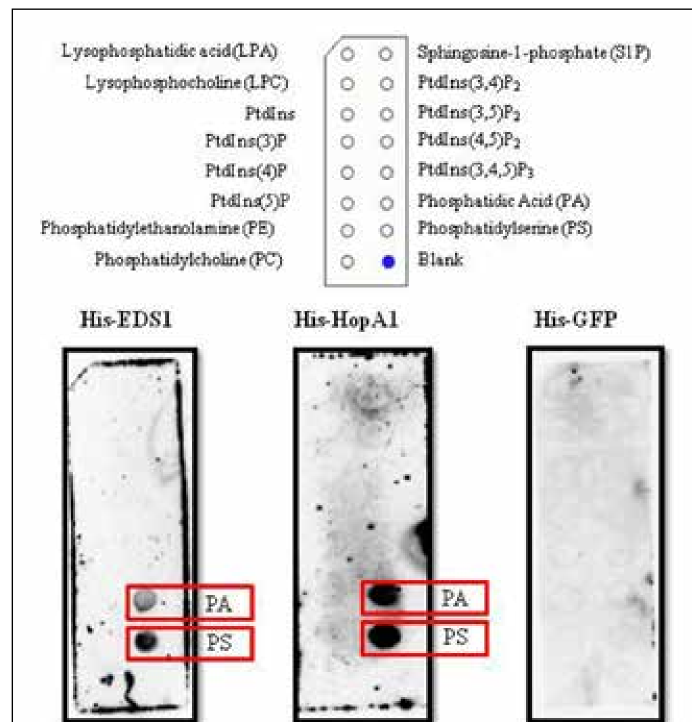

Figure 24: EDS1 and HopA1 bind to overlapping lipids in in vitro lipid-binding assays. E. coli expressed and purified His-EDS1, His-HopA1 and His-GFP were tested by overlay blots using lipid-coated strips. Binding was detected by immunoblot with anti-His antibodies.

Interestingly, both PA and PS form distinct membrane microdomains on endosomes and endoplasmic reticulum (ER) and may harbor the SRFR1-associated resistasome complex. Indeed, in preliminary tests co-localization of SRFR1 with endosomal and ER markers was detected. Thus, the results identify a lipid-associating component of the SRFR1-resistasome. It had earlier been reported that the pathogenic effector HopA1 targets and disrupts EDS1 associations in the SRFR1-resistasome. To determine the mode of HopA1 function, the lipid-binding propensity of HopA1 was also tested. Surprisingly, similar to EDS1, HopA1 also bound to PA and PS (fig. 24). Although the functional relevance of these associations needs to be further determined, nevertheless an overlap of lipid-binding properties closely link EDS1 as a HopA1 target. In plant defenses, both PA and PS have been implicated as potent defense signaling mediators. The association of EDS1 with these lipids likely reflects a coupling of defense proteins with signal transducers whereas HopA1 interactions may signify its virulence function in suppressing defense by binding to immune signaling messengers.

The overlapping propensity of EDS1 and HopA1 to bind selective lipids likely connects immune modulators and pathogen effectors to signaling messengers. Incidentally, both EDS1 and HopA1 have /b hydrolase-like motifs, however their activity remains undetermined. Domain mapping of lipid-binding residues will identify whether lipase motifs overlap with lipid-binding functions. Further on, we are generating transgenic Arabidopsis lines expressing chemical-inducible HopA1, which will be subsequently used to identify virulence targets of the effector.

Post-translational modifications (PTMs) by SUMO regulate functions of central defense modulators

The mode of assembly of the SRFR1-associated resistasome and its interactions with other defense regulators remain unidentified. Considering the presence of multiple disordered domains and its close proximity to SUMOylation motifs, it can be speculated that the importance of SUMO (Small Ubiquitin-like Modifier) in SRFR1 functions as a negative regulator. In Arabidopsis, 4 SUMO isoforms are expressed. Previous literature suggests that SUMO1 and SUMO2 are redundant in function, whereas SUMO3, a slightly diverged isoform may play distinct roles. The role of SUMO5 remains to be elucidated. It was noted that global SUMOylation by SUMO1/2 or SUMO3 is enhanced in the auto-active srfr1-4 plants (fig. 25).

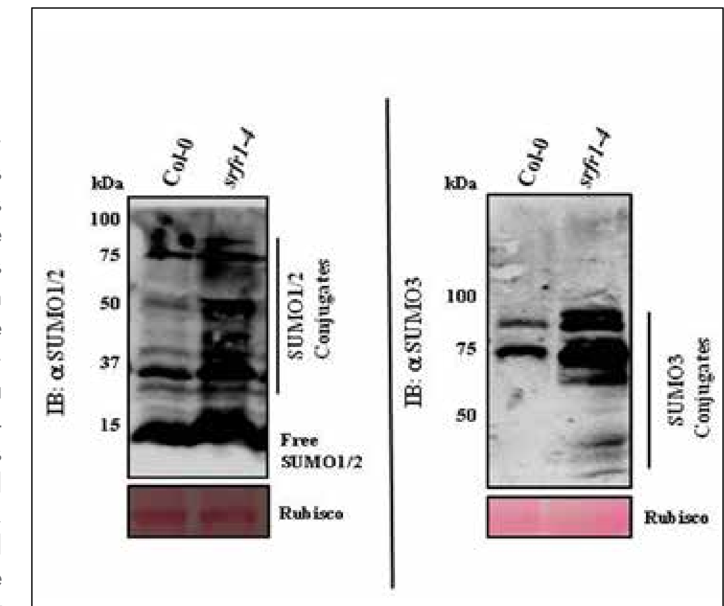

Figure 25: SUMOylation profile in srfr1-4. Total protein extracts from wild-type (Col-0) and srfr1-4 plants were immunoblotted with anti-SUMO1/2 (left panel) or anti-SUMO3 (right panel) antibodies.

Surprisingly, qPCR analysis in srfr1-4 revealed that SUM1 and SUM2 transcripts are modestly downregulated whereas SUM3 shows significant upregulation. Therefore, to account for increased SUMOylation, the transcript levels of other SUMOylation-related genes were investigated. Transcripts of several of the SUMOylating enzymes such as SAEs and SCE1 were upregulated in srfr1-4. Simultaneously, significant down-regulation of several de-SUMOylating enzymes (ESD4 and ELS1) in srfr1-4 plants was also detected. Taken together, the data suggests that a global increase in SUMOylation in srfr1-4 is achieved by coordinated expression levels of these genes.

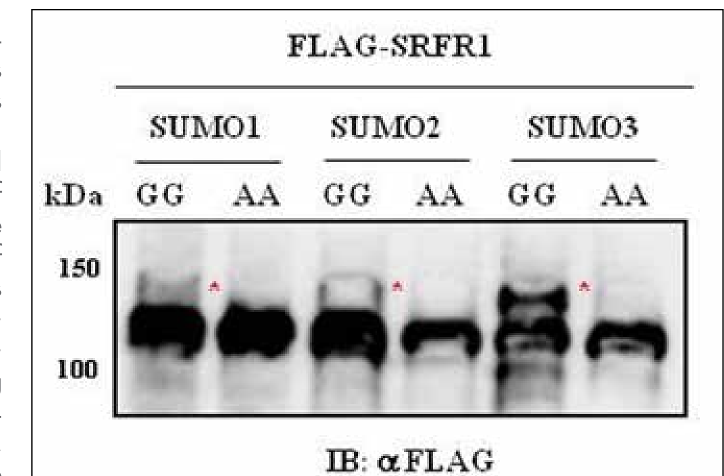

Figure 26: FLAG-epitope tagged SRFR1 was co-expressed in E. coli SUMOylation system with SUMO1, SUMO2 or SUMO3 (right panel). SUMOylation proficient and deficient SUMOs are indicated with GG and AA, respectively. Total proteins were immunoblotted with anti-FLAG antibodies. SUMOylated FLAG-SRFR1 is indicated with asterisks.

The contributions of SUM1, SUM2 and SUM3 in the immune functions were subsequently explored. In pathogenicity assays, mutations in SUM1 (sum1-1) or SUM2 (sum2-1) caused elevated defenses that conferred enhanced resistance to the corresponding mutants towards P. syringae. Levels of several defense markers in the sum1-1 and sum2-1 plants were also elevated. Contrastingly, sum3-1 plants exhibited enhanced susceptibility to P. syringae. This observation suggests an antagonistic role of SUMO3 to SUMO1/2 functions. When tested for SUMOylation-potency in an E. coli SUMOylation system, intriguingly SRFR1 was indeed confirmed as a SUMOylation target of all three SUMOs (fig. 26). Ongoing work is aimed at testing whether SRFR1 functions are influenced by the type of SUMO isoform it is conjugated to.

SUMOylation sites in SRFR1 are being mapped to identify specific lysine residues that attach SUMOs. These site(s) will be either sequentially or combinatorially mutagenized to determine their importance in SRFR1 functions. Whether SRFR1 functions are affected in the sum1-1 or sum3-1 is also being investigated.

Modulation of Host Immunity and Nutrient Allocation by a Biotrophic Pathogen

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Powdery mildew is a significant fungal disease of legumes, which represent major food crops cultivated and consumed in India. The main goal of this research program is to identify targets for biotechnological intervention that will help develop effective and eco-friendly disease control in important crop legumes. Interactions between the pea powdery mildew Erysiphe pisi and its legume hosts are studied to identify plant factors that promote successful fungal colonization, plant defense responses that restrict fungal growth without an associated loss of yield, and the fungal molecules that promote pathogenesis.

Powdery mildews (PM) are significant fungal pathogens of legumes that cause yield losses of ~25-60% in agronomically important food legumes such as pea, lentils and mung bean. PM fungi depend entirely on living host plants for their survival and are hence considered to be true obligate biotrophs. They infect aerial parts of the plant and form a white powdery coating on infected leaves, stems and fruits. The ephemeral nature of R-gene-mediated resistance and the harmful effects of fungicides that are traditionally used to control the disease have fueled the search for innovative & sustainable disease management solutions. Further, the high degree of host specialization exhibited by these fungi has prevented the direct translation of information gleaned from other well-studied plant-PM pathosystems (e.g. Arabidopsis-PM and barley-PM) to legumes. Therefore, to develop innovative biotechnological strategies to limit PM disease progression on legumes, this program aims to elucidate the molecular mechanisms underlying legume-PM interactions using the pea PM Erysiphe pisi and its legume hosts, Medicago truncatula and pea, as model systems. The major goal of this program is to develop legume crops with broad-spectrum and durable resistance to the powdery mildew pathogen. For this, the aim is to identify host resistance and susceptibility factors that impact the biotrophic growth of the pathogen. Concurrently, the study also aims to identify and characterize fungal effector molecules that promote host colonization. It is envisaged that targeting a combination of factors would result in dramatically reduced pathogen proliferation and would contribute to durable crop resistance that is less likely to be rapidly overcome by pathogen counter-evolution.

Investigation of host resistance mechanisms against PM infection

To uncover PM resistance factors, transcriptome profiling of a mixed host-pathogen RNA sample was performed using an RNASeq approach. Briefly, leaves from three-week-old resistant (R) and susceptible (S) M. truncatula genotypes infected with a moderate dose of E. pisi were harvested at 1 day post inoculation (dpi). RNA extracted from uninfected and infected tissues were converted to cDNA libraries and sequenced using the Illumina HiSeq platform. Deep sequencing of two independent biological replicates yielded ~ 82-133 million high-quality (HQ) reads. HQ reads were aligned to the merged M. truncatula and E. pisi genomes and assembled using the Trinity Assembler. The primary assembly provided 43840 transcripts with an N50 Contig size of 1,581 bp. The assembled transcripts were annotated by mapping the non-redundant sequences to the M. truncatula protein database available at JCVI (medicagogenome.org) and the NCBI (ncbi.nlm.nih.gov) plant Refseq

protein database using BLASTX searches. In the analysis, 35,466 (81%) transcripts out of 43840 were annotated according to BLASTX scores e-value cutoff of 1e-

3 and minimum query coverage of 50%. Differential gene expression analysis of M. truncatula genes revealed that 284 genes were upregulated and 33 genes were downregulated by at least 2-fold ($p<0.05$) in response to E. pisi inoculation in the R genotype. Similarly, 214 were upregulated and 33 downregulated at least 2-fold ($p<0.05$) in response to E. pisi inoculation in the S genotype. Surprisingly, a number of transcripts exhibited sample-specific expression; 598 and 654 transcripts were specifically expressed only in infected R and S genotypes, respectively. This indicates that in addition to differential expression, active opening of the chromatin for initiation of transcription of particular subsets of genes is triggered upon E. pisi infection. Gene Ontology (GO) enrichment analysis was performed on differentially expressed genes (DEGs) of both R and S samples to identify functional categories enriched upon E. pisi inoculation (fig. 27). Top GO biological process categories enriched in R DEGs belonged to defense response, regulation of defense response, response to biotic stimuli, phosphorylation, cell wall catabolism, and flavonoid metabolism, indicating that activation of signaling and defense mechanisms is a strategy used by the R host to restrict fungal growth. By contrast, GO biological process enriched categories in S DEGs largely belonged to metabolism & transport. This is consistent with the obligate biotrophic lifestyle of the PM pathogen, which is known to modulate host metabolism to fuel its own growth and reproduction. GO cellular component enriched categories in R DEGs include various membrane organelles, the cell wall and the nucleus. These components have previously been shown to be involved in restricting fungal penetration by forming physical barriers or transporting antimicrobial compounds to the site of attack. Interestingly, these categories were not enriched in the S DEGs. Differences in GO molecular function enriched categories were also observed between R and S DEGs. For example, chitinase activity was enriched in R DEGs whereas amino acid transport activity was enriched in S DEGs. Degradation of the chitin-rich fungal cell wall may prevent pathogen growth in the R host whereas the pathogen may activate host amino acid transporters for uptake into the S host.

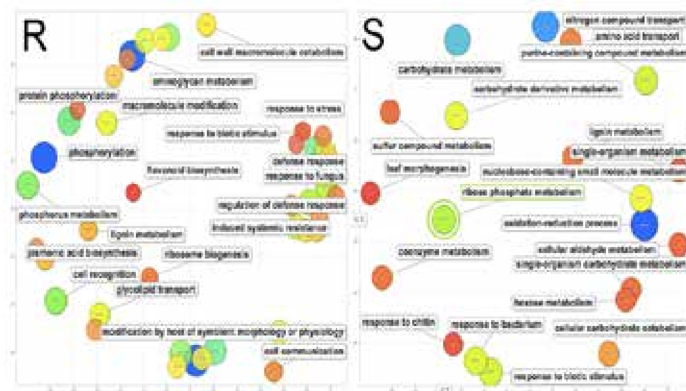

Figure 27. Gene Ontology enrichment analysis of DEGs. Revigo plots summarizing GO Biological process terms for R and S datasets.

Identification of Erysiphe pisi effector candidates

PM fungi secrete an arsenal of effectors into the host, which are capable of modulating host metabolism and cellular architecture to promote host colonization while suppressing host immune responses. Identification of these effectors and their host targets is critical for unravelling mechanisms of pathogenicity as well as discovering novel aspects of plant immunity. PM fungi form specialized feeding structures within host epidermal cells, termed haustoria, which serve as the primary sites of effector delivery. To identify candidate effectors, haustorial complexes were isolated from pea plants infected with E. pisi, followed by in-depth sequencing of haustorial cDNAs using the Illumina HiSeq platform. A combination of genome-guided and de novo-based approaches led to the assembly of ~ 30,000 unique haustorial transcripts. To identify secretory proteins, predicted open reading frames (ORFs) with genuine start codons were scanned for the presence of secretion signals using SignalP3.0 (CBS). ORFs with signal peptides were passed through a TMHMM prediction tool to remove proteins with transmembrane domains. Using this approach, approximately 500 secretory proteins were predicted. Of these, 88 were found to possess the conserved Y/F/W-x-C motif, which is typically present downstream of the signal peptide cleavage site in PM effectors. Furthermore, these 88 proteins do not show any homology to proteins in the NCBI nr database outside the PMs and were thus considered as candidate effector proteins of E. pisi (EpEFs). The evolutionary history of EpEFs was inferred using the neighbor-joining (NJ) method, and distances were computed using the p-distance method with MEGAv7.0. Based on the NJ phylogenetic tree, 88 EpEFs grouped into 6 distinct clusters (fig. 28). The overall bootstrap support of the NJ tree was low, but this may be attributed to the low sequence relatedness, which is a characteristic of PM effectors. A comparative analysis with other sequenced PM species revealed that 19/88 EpEFs were homologous to effector candidates of the closely-related grape PM Erysiphe necator, whereas only 1/88 and 0/88 were homologous to that of more distantly-related Arabidopsis and barley PMs, respectively.

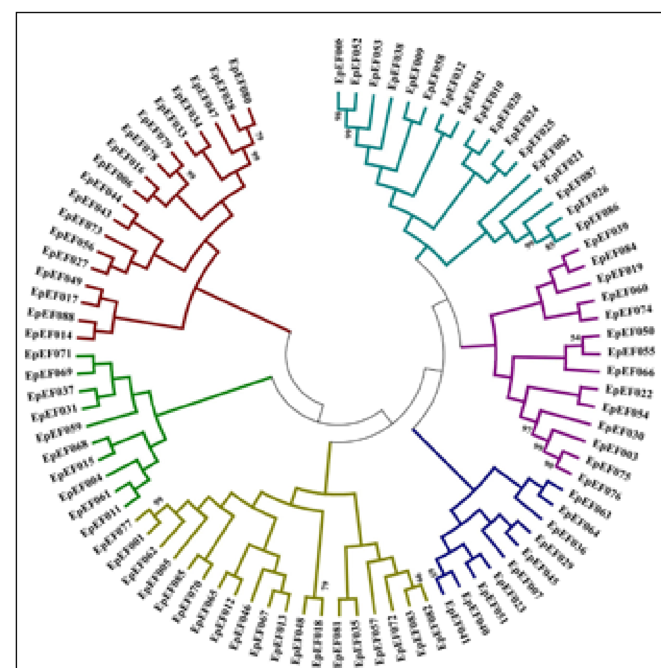
Figure 28. The unrooted NJ tree showing evolutionary relatedness of the 88 EpEFs from this study. Bootstrap support values greater than 40% are shown next to the branches.

Elucidation of sugar-related mechanisms impacting PM growth at the host-biotroph interface

PM infection typically creates an additional sink in infected tissues, which can lead to significant changes in sugar transport and partitioning within the plant. Specifically, the fungal haustorium creates an apoplastic interface between the pathogen and the host, through which sugars released from the host are absorbed by the fungus. Early studies that measured sugar levels in apoplastic washes and radioisotope-labeled carbon uptake into PM-infected leaf discs and/or mycelia indicated that glucose is the primary carbon source transported and metabolized by the fungus. This was supported by the fact that the activity of cell wall invertases (CwINV), which break down sucrose into glucose and fructose, and levels of reducing sugars were higher in PM-infected leaves. Hexoses generated by cleavage of apoplastic sucrose will then have to be transported into the haustorium-containing epidermal cell via plant hexose transporters before making their way into the haustorium. Since a hexose transporter (STP family member) and cell wall invertase (CwINV) were previously reported to be induced in response to other PMs, the expression of M. truncatula orthologs of these genes in response to E. pisi infection in S and R genotypes was examined. E. pisi is able to form haustoria and reproduce on the S genotype, whereas its growth is restricted to the appressorium stage on the R genotype. In the S genotype, MtSTP expression was initially repressed/unchanged at 1 and 3 dpi, but increased ~3-fold at 5 dpi (fig. 29A). Similarly, MtCwINV showed very low expression at 1 dpi but increased up to 2-fold at 3 and 5 dpi (fig. 29B). In contrast, in the R genotype, MtSTP13 was induced ~10-fold at 1 dpi, and further increased to ~20-fold at 3 and 5 dpi (fig. 29A). Similarly, MtCwINV showed a 5-10-fold induction at all time points tested in R (fig. 29B). It is well known that sugars can act as signaling molecules to induce defense responses in addition to serving as nutrients. The rapid and sustained induction of MtSTP and MtCwINV genes in the R host that does not support haustorial formation suggests that infection-dependent repartitioning of hexoses may trigger host defenses that serve to restrict pathogen growth. In agreement with this hypothesis, this response appeared to be delayed and of lower magnitude in the S host. To check whether these genes exhibit infection site-specific expression in the S host, transcript abundance was quantified from infection sites isolated using laser capture microdissection (fig. 29C). Interestingly, in contrast to the whole leaf expression data, expression of both MtSTP and CwINV increased by almost 12-fold at the E. pisi infection site at 3 dpi in the S host (fig. 29D). Whether this infection site-specific expression is a reflection of the pathogen hijacking a plant transport machinery for its own benefit or the plant competing with the pathogen for hexoses at the plant-biotroph interface requires further investigation.

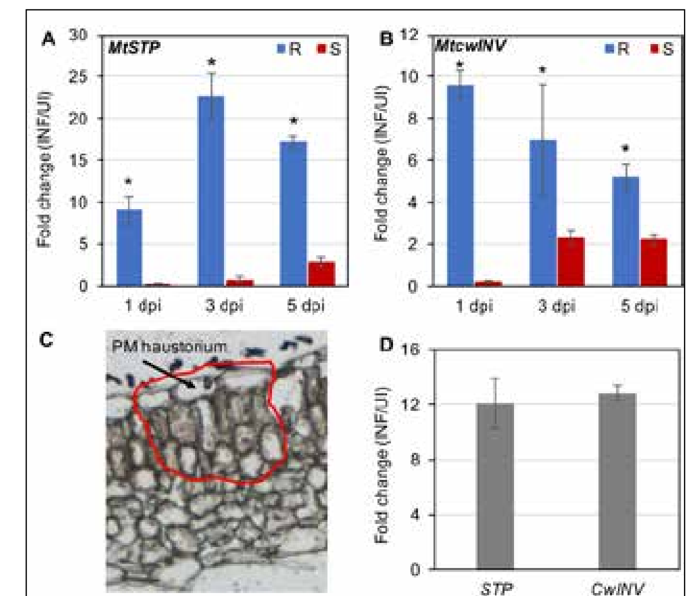

Figure 29: Differential regulation of MtSTP and MtCwINV upon PM infection in R and S M. truncatula genotypes. qPCR expression analysis of MtSTP (A) and MtCWINV (B) at 1, 3 and 5 dpi. Laser microdissection-assisted isolation of PM infection sites(outlined in red) (C) followed by site-specific expression analysis (D) at 3 dpi in the S genotype. UBQ was used as the endogenous control. *p 0.05 based on student's t-test.

As a follow-up to the transcriptome profiling study, a genome-wide miRNA: mRNA integrome analysis will be performed to construct gene regulatory networks differentiating PM resistant and susceptibility traits. To characterize the function of EpEF candidates in PM pathogenesis and identify their host targets, in planta localization, pathogen susceptibility testing and yeast two hybrid assays will be performed in pea and/or M. truncatula. To functionally characterize the role of MtSTP in PM resistance, mutants and/or silenced lines will be developed in M. truncatula and/or pea, and tested for altered PM phenotypes. Further, subcellular localization of this transporter and substrate specificity will be determined using reporter constructs and yeast mutant assays respectively.

Understanding taste and its modulation using Drosophila melanogaster as a model system

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Taste is extremely important for all the organisms to evaluate and choose foods that are rich in calories and avoid bitter compounds that may be toxic. Like humans, Drosophila flies can differentiate various taste stimuli. By exploiting the taste system of flies, the research program aims to understand how flies make feeding decisions and how the taste information is wired and processed in the brain. From both health and agricultural viewpoints, a greater understanding of the neuronal pathways that regulate taste behaviors of insects and how they are modulated can greatly improve the quality of human life.

Using the fruit fly Drosophila melanogaster, this research program is trying to understand how insects make feeding decisions and how the taste information is wired in the brain. This involves identifying unknown neuronal taste circuits in the brain, physiological state and factors that act on the taste cells and circuits, and modulate the taste behavior.

For all animals, the sense of taste provides the ability to evaluate the quality of food sources and promote ingestion of nutritious substances and discourages consumption of harmful substances. The gustatory system of Drosophila is being studied to understand the taste circuits and their modulation to achieve the main objectives of how specific neuronal circuits influence-feeding behaviors, identifying the neuronal pathways that regulate satiety and how taste information at the periphery and central nervous system is modulated. Disease carrying and crop destroying insects use their senses of taste and smell to find hosts and food. Insect-borne diseases such as malaria, dengue fever and Chikungunya are transmitted via feeding behaviors. The results from simple model systems like Drosophila could potentially be applied to safe and cost effective pest control by improving insect trapping strategies and thus reduce pathogen transmission by insects and greatly benefit the agricultural industry and society as a whole.

Understanding how specific taste neuronal circuits influence feeding behaviors

The nervous system evaluates the internal metabolic state and external chemosensory information to control hunger and satiety. The proper function of this complex physiological system is essential to balance nutrient intake, maintain stable body weight and regulate metabolism. When the balance between hunger and satiety is perturbed, food intake is misregulated, leading to excessive or insufficient eating. Understanding the neural mechanisms that motivate us to eat and tell us when to stop is important because of the increasing rates of obesity, diabetes and cardiovascular disease in our society. In humans, abnormal nutrient consumption causes metabolic conditions like obesity (causing 3 million deaths/ year) and eating disorders. Despite this burden on society, there is currently a lack of enough knowledge about the neuronal pathways, circuits and genes that regulate appetite.

By exploiting the gustatory system of the flies, the study is interested in understanding how sensory information is processed by the brain to give rise to specific taste

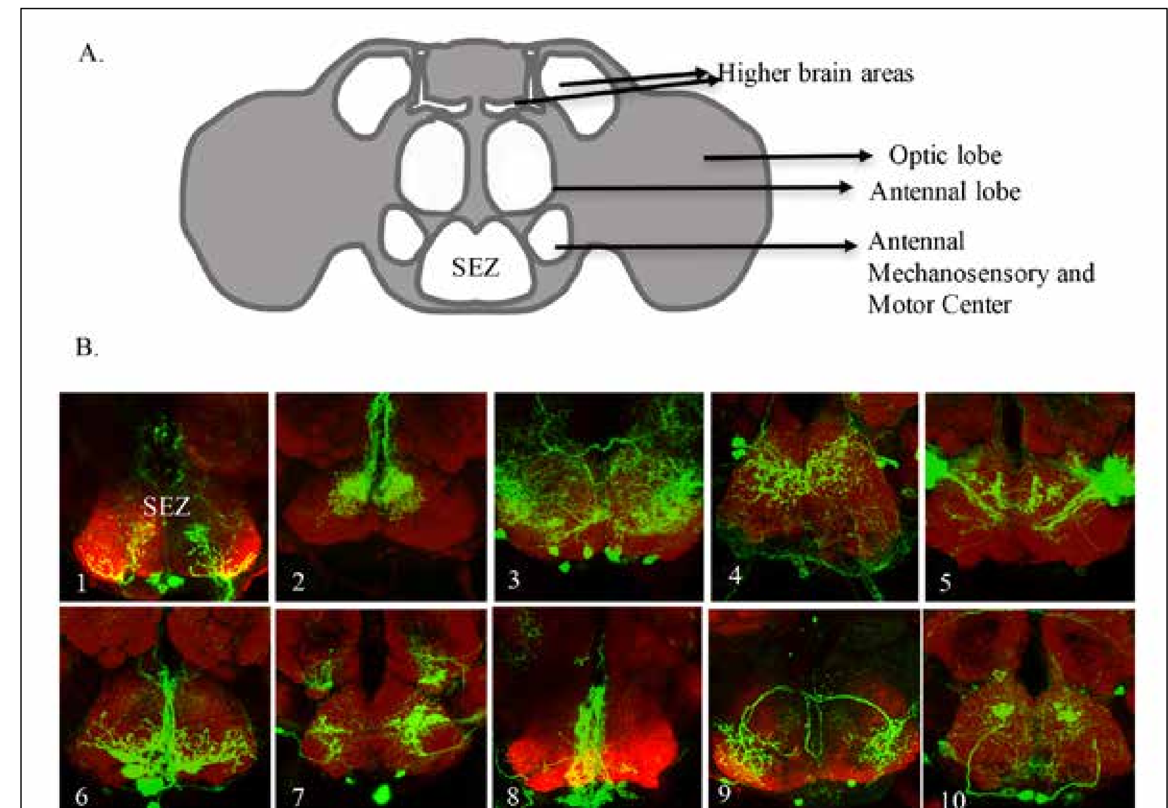

Figure 30: (A) Adult Drosophila brain showing subesophageal zone (SEZ). SEZ is a first relay for taste information. (B) Identifying neuronal pathways involved in the feeding behaviors. Expression pattern of some of the newly identified neurons in the SEZ of the adult fly brain. GAL4 driven expression of UAS-mCD8::GFP in the SEZ for different GAL4 drivers (visualized with anti-GFP green). For all brain images, neuropil is stained with anti-nc82 (red). Various numbers indicate different GAL4 driver lines marking different set of neurons obtained from Janelia farm GAL4 collection.

behaviors. Specifically, the interest is in understanding how the taste information is wired in the brain and how it is modulated by intrinsic and extrinsic factors. Drosophila can sense the same taste stimuli as mammals, including sugars, water, salts, acids, alcohols and bitter tastes. These compounds facilitate acceptance or avoidance behaviors, although innate taste behaviors may be modified by learning and experience. The simplicity of ligands and behaviors, along with experimental strategies involving molecular, genetic, calcium imaging and electrophysiological approaches allows one to examine taste processing from sensory input to motor output in a system that can also be modified by learning. The study will attempt to dissect the taste neural circuits that convey taste information to the brain and are involved in simple feeding behaviors like acceptance or rejection of food.

To understand how the taste information is processed and how feeding is regulated in flies, various promoter GAL4 lines with the GFP maker are being screened to identify these circuits in the brain. Based on their expression pattern in the brain, many neurons that are involved in the appetitive behavior and are potential candidates for sugar sensing have been identified (fig. 30A and B). These unmapped taste neural circuits will be delineated with cellular resolution, using molecular genetic approaches to label, activate and silence these neurons, and electrophysiological and calcium imaging approaches to monitor taste-induced activity. These studies will aim to examine how the brain processes taste information to allow for stereotyped behavior, behavioral plasticity, taste learning and memory, and individual variation. Identification of various neurons will provide valuable insight into the neural architecture of appetitive and aversive circuits.

Effect of high salt feeding on taste preferences

Salt (NaCl) is an essential component of our diets. Presence of salt makes food more palatable than the same food with no salt. The right and small amount of salt is essential for our health. Data from the literature suggests that adults need less than 1 gram per day and children need even less. In India, the general salt consumption is approximately 8.0 g of salt per day, far more than we need, putting us at risk of various health problems like blood pressure. Raised blood pressure (hypertension) is the major factor that causes strokes, heart failure and heart attacks, the leading causes of death and disability worldwide. There is also increasing evidence of a link between high salt intake and stomach cancer, osteoporosis, obesity, kidney stones, kidney disease and vascular dementia and water retention. Salt can also exacerbate the symptoms of asthma, Ménière's disease

and diabetes. A high salt diet can cause calcium to be lost from bones and excreted in the urine, making bones weak and easily broken.

Recent evidence indicates that an acceptable salt substitute is unlikely, an understanding of the neural circuits, taste receptors, behavioral and sensory factors involved in maintaining high salt preference is a prerequisite to successful programs aimed at reducing intake. Although little evidence exists for a genetic determination of individual differences in consumption and preferred level of salt, more research in this area is necessary. Considerable data support the view that the optimal level of salt in the diet is determined in part by the level an individual is currently consuming; increasing or decreasing customary salt intake, as long as the salt is tasted, increases or decreases the preferred level of salt in food. Although these data are consistent with a hypothesis that optimal salt preferences are learned, other data, from both animal models and human developmental studies, suggest that salt preference has an innate component. Liking for salt, similar to liking for sweets, has an innate basis that can be modified by individual experience. To understand how early experience with high salt diets may have a long-term impact on preferred salt levels or other taste modalities, the study is looking into the neural circuits and molecular mechanisms of high salt feeding in Drosophila which has not been explored so far.

To understand how high salt preference affects the feeding behavior and change preferences for other taste modalities, two choice feeding assays are being used with edible red and blue dyes to monitor what and how much flies are eating (fig. 31A). Based on what flies choose to eat in dark boxes in an unbiased manner [red, blue, purple (if eating both) or none- color of their abdomen], it was observed that flies feeding on high salt previously, have low preference for other taste categories.

Preliminary results demonstrate that flies like to feed on moderate amount of salt (50mM) and show aversive behavior towards salt as the salt concentration increases (fig. 31B). Feeding on high salt added in the normal diet (200mM) has an impact on other taste categories. Flies eating high salt (for 4-5 days) added with the media in the culture tubes, show less preference for sugars but have high preferences for high salt in the two choice feeding assays. They show no change in their feeding preferences for other categories of salts. Our results suggest that flies on a high salt diet like to feed on single dominant taste like sour or chilly alone but not on mixtures, for example sour + salt; sour + sugar etc. Flies eating high salt laid far less number of eggs, showed developmental delays and have shorter life span as compared to flies feeding on normal media (fig. 32A and 32B).

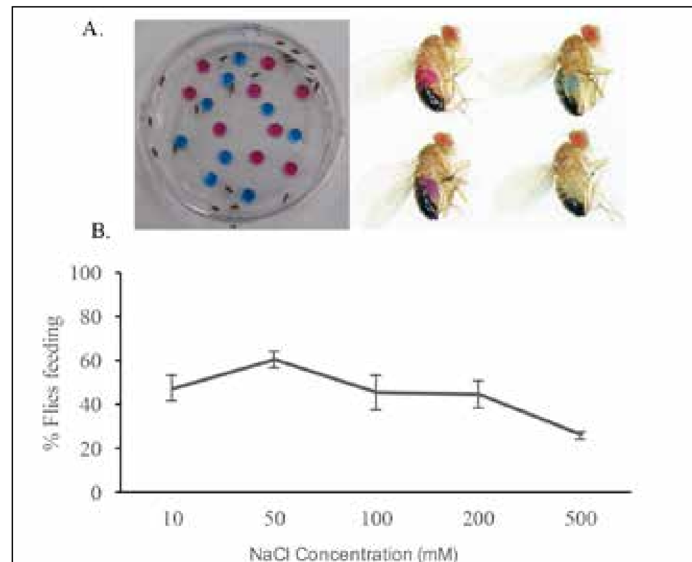

Figure 31: (A) Two choice feeding assay for adult flies. Red spots on the feeding plate is salt mixed with dye and agar. Blue are control dots with agar and dye only. Flies are scored (abdomen color) based on what they prefer to eat during the assay (2hrs time) (B) Dose response curve for salt (N=6 plates for each concentration). Wild type flies (CsBz) show highest attraction towards salt at 50mM concentration and aversion at high salt concentrations.

In Drosophila Ir76b, a sodium (Na+) permeable channel has been shown to be required for sensing low salt and bears no relationship to ENaC channels that are required for sensing low salt in mice. The identity of the high salt receptor is still mysterious. The study is in the process of ascertaining where and how high salt feeding modulates the taste behavior to other taste categories.

Understanding the mechanism of high salt feeding and its effect on feeding behavior, longevity, mating and egg laying behavior in insects like Drosophila will help in preparing inexpensive and effective pesticidal salt baits for pest control.

Understanding age related changes in taste preferences

Both smell and taste play vital roles in food enjoyment and safety. A delightful meal or pleasant smell can improve social interaction and enjoyment of life. In mammalian model systems, various groups have reported that the number of taste buds decreases with age. It has also been suggested that sensitivity to the five main tastes often declines after age 60 in humans. In addition, our mouth produces less saliva as we age. This can cause dry mouth, which can affect the sense of taste. Decreased taste and smell can lead to less interest, diminished appetite and no enjoyment while eating. Using Drosophila, the study is trying to understand the effects of aging and diseased conditions on taste behavior. In preliminary two choice feeding assays, less sugar feeding was observed in aged flies (~35 days old) compared to young flies (~7 days old) even to high concentration of sugars, which is a main source of energy for animals. Understanding the age-related factors affecting taste can help humans prepare to accept change, adapt, and be aware of potential hazards and help in aging gracefully with changed heathy eating habits.

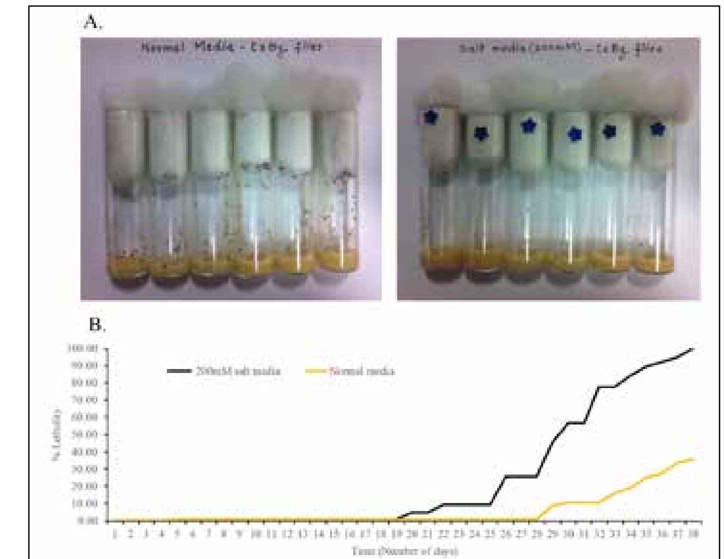

Figure 32: (A) Wild type flies (CsBz) growing on high salt media show developmental delay. Flies eating high salt throughout the development take more time to eclose at 250C (14-15 days) and grow slower than control flies on normal media (10-11 days) (N=6 tubes in both conditions). (B) Wild type flies feeding on high salt have shorter life span. Freshly eclose wild type flies were transferred on high salt mixed with normal media and on regular fly media (flies transferred regularly every 3rd day on fresh media conditions during the assay). Percent lethality was calculated on each day (N=6 tubes each condition, each tube contained 20 flies-10 males and 10 females). Adult flies feeding on high salt media die early (day 38) compared to control normal media flies (60-90 days).

In the future, investigations on the role of different physiological states like hunger and thirst that might alter the responses of newly identified candidate neurons and various gustatory receptors will be undertaken. Feeding responses of flies will be assessed under food and water deprivation conditions. The results will be compared by performing calcium imaging on these neurons in thirsty, fed and starved flies by stimulating the labellum or legs to various taste modalities. For all the identified neuronal circuits, their synaptic connectivity with the peripheral receptors and their neuromodulator identity will be tested by immunohistochemistry by using antibodies specific for different neurotransmitters and neuromodulators.

It has been shown that dopamine signaling plays a critical role in reducing behavioral thresholds to sucrose upon starvation. To test whether the activity of candidate neurons is modulated via dopamine or by any other neuromodulator signaling, calcium activity in the candidate neuronal lines will be recorded by expressing GCaMP (genetic calcium indicator construct in flies) either by feeding flies with food mixed with the neuromodulators or by expressing the RNAi of various neuromodulators with the GCaMP.

While enjoying food, it is essential that one should know when to stop when one is full. Metabolic conditions and eating disorders are affecting millions of people every year. Increased consumption of sweet products is a growing concern with medical authorities and it has been linked to the rising incidents of diabetes and obesity all over the world. Hence, it is essential to balance the nutrient intake and maintain stable body weight to regulate metabolism. The study is interested in exploring how hunger and satiety are achieved by identifying pathways, neurons and genes involved and in relating the findings to homologous mammalian genes with similar functions to discover conserved pathways that regulate hunger and satiety.

Academic Activities

Academic Activities

Doctor of Philosophy (PhD) Degree Programme

As per articles of RCB Act 2016, a multidisciplinary PhD programme has been instituted for students who have completed Master's degree in any area of science and technology, or a Bachelor of Medicine, Bachelor of Surgery degree, or an equivalent degree from a university or institution recognized by the Academic Committee. In addition to the qualifications specified above, the candidate for admission shall qualify a national level test for pursuing PhD programme and such test include a doctoral fellowship or admission eligibility test conducted at the national level by the UGC, or CSIR, or ICMR, or DST, or DBT, or any other Government-recognized agency. The candidates, who fulfill both the criteria (specified qualifications in addition to qualifying national level test), shall be admitted to the PhD programme after a written test or an interview or both (conducted by RCB) as may be specified by the Academic Committee. This year 25 students registered for the PhD degree programme.

Doctor of Philosophy (Integrated) Degree Programme

The RCB Act 2016 has empowered the Centre to grant an integrated MSc-PhD program in Biotechnology. The minimum qualification for admission to the Doctor of Philosophy (Integrated) Programme in the Regional Center shall be the Bachelor's degree in Science or Engineering or Medicine or an equivalent degree. Admission of the applicants to the PhD (Integrated) Programme shall be by a written test or an interview or both, as approved by the Board of Studies. Applications for this programme are currently open and the programme will commence from the academic session 2018-19.

Seminars by Visiting Scientists

Date	Speaker	Title
September 8, 2017	Dr. Prateek Tripathi, The Scripps Research Institute La Jolla, CA 92037	Understanding the mechanistic links between the circadian clock and plant metabolism for crop improvement
August 16, 2017	Dr. Geetanjali Chawla Department of Biology Indiana University, USA	MicroRNA pathways and their role in aging and neurodegeneration in Drosophila
June 14, 2017	Dr. Geeta Ram New York University School of Medicine (NYSOM), New York	The role of staphylococcal pathogenicity islands (SaPIs) in the adaptation and virulence of Staphylococcus aureus
May 31, 2017	Dr. Nidhi Adlakha JNU, New Delhi	Path to Product-Development of microbial cell factories for innovative bioproduction
May 25, 2017	Dr. Tarun Jain	Application of Computational Techniques in Drug Discovery
April 27, 2017	Dr. Arun Khatri Department of Medicine, The University of Chicago, Chicago, IL 60637	Head and Neck Cance - Integrative Genomic Analysis and Next Steps in Immunotherapy
April 21, 2017	Dr. Malay Patra Department of Chemistry, University of Zurich, Zurich, Switzerland	Glyco-Conjugation Strategy for Targeted Delivery of Platinum Anticancer Drugs
April 07, 2017	Dr. Sabari Sankar Thirupathy University of Wisconsin-Madison	Genomic instability at the crossroads of replication and transcription
March 30, 2017	Dr. Ellora Sen National Brain Research Centre, Manesar	Metabolic storms: feeding the tumor black hole
March 27, 2017	Dr. Charu Lata, Scientist Division of Plant-Microbe Interactions CSIR-National Botanical Research Institute Lucknow, India.	Exploration of genetic and genomic resources in millet models for drought stress tolerance and nutritional quality traits
March 22, 2017	Dr. Hathi Ram	Regulation of lateral organ initiation by transcription factors (TFs) involved in dorso-ventral (DV) patterning in Arabidopsis
February 23, 2017	Dr. Prem Singh Kaushal Wadsworth Center, NYS-Department of Health, Albany, NY, 12201 USA.	Cryo-electron microscopy (cryo-EM) studies of the ribonucleoprotein complexes: group II intron and ribosomes

Seminars by Visiting Scientists

Date	Speaker	Title
February 22, 2017	Prof. Maria Elena Bottazzi Baylor College of Medicine Houston, Texas, United States	Global Health Technologies: Innovative and multidisciplinary research & development to combat neglected tropical diseases
December 29, 2016	Dr. Pankaj Kumar Arora Rohilkhand University, Bareilly, India	Current Scenario and Future Directions of Biodegradation and Bacterial Taxonomy in India
December 16, 2016	Dr. Sambasivam Periyannan Research Scientist, CSIRO Australia	Novel strategies for cloning defense genes in plants
December 08, 2016	Dr. Malancha Ta IISER-Kolkata	Assessing the Effectiveness of Umbilical cord-derived mesenchymal Stem Cell under Conditions of Ischemia
December 08, 2016	Dr. Pratap C. Naha postdoctoral fellow at university of Pennsylvania Philadelphia, PA, USA	Novel nanoparticle contrast agents for X-ray imaging (computed tomography and dual-enerdy mammography)
October 28, 2016	Dr. Anjil Kumar Srivastava Durham University, UK	SUMOylation: Fine tuning of biotic and abiotic stress responses in plants
October 26, 2016	Prof. Jean Francois Riou Director, Laboratory of Structure and Genome Instability Museum National d'Histoire naturelle, Paris	G Quardruplex Ligands are more than simple telomere targeting agents
October 18, 2016	Dr. Pankaj Chauhan Johna Hopkins School of Medicine Baltimore MD, USA	Structure-based Drug Designing Against Infectious Diseases

Scientific Events at RCB

An International Symposium-cum-workshop on 'Structural proteomics of Macromolecular Complexes using X-ray crystallography and Mass spectrometry" was organized at the Regional Centre for Biotechnology from 18th to 20th December 2016 that brought together eminent scientists, researchers from finest institutions from around the globe to exchange ideas. The meeting focused primarily on progressive developments in macromolecular crystallography, NMR, cryo-EM and Mass spectrometry as sources for structure determination. The three-day event featured lectures and workshop tutorials by distinguished experts from India and overseas.

The workshop was attended by faculty, post-docs and students from India, with total participants being 20. There were four international speakers which included Philip Andrew (University of Michigan, USA), David E. Wemmer (University of California, Berkeley, USA), Dr. Ganesh Anand (National University of Singapore, Singapore) and Dr. Rajendra Agrawal (Wadsworth Center, New York State Dept. of Health and State University of New York, USA). The speakers from India included Dr. Dinakar M. Salunke (ICGEB, New Delhi), Prof. S. Ramaswamy (inStem, Bangalore), Prof. Jayant Udgaonkar (National Centre for Biological Sciences, Bangalore), Dr. R. P. Roy (National Institute of Immunology, New Delhi), Dr. Deepak T Nair (Regional Centre for Biotechnology, Faridabad), Dr. Saikrishnan Kayrat, (IISER Pune), Dr. Neel Sarovar Bhavesh (ICGEB, New Delhi) and Dr. Amit Mandal (St. John's Research Institute, Bangalore).

The three-day event included a workshop on X-ray crystallography and Mass spectrometry, which was conducted by scientists from India and abroad where hands-on training was provided to students in crystallization, molecular interaction studies and mass spectrometry. The event was sponsored by SERB, ICON Analytical, FEI, PALL forte bio, Amitlabs, and Bruker. The event was a huge success and a number of attendees suggested that it should be held once every two years.

The first RCB-AIST mini-symposium on "Cellular Mechanisms in Health and Disease", as part of the DBT-AIST laboratory was held at RCB on February 2nd, 2017. This was a one-day event with enthusiastic participation from students, post-doctoral fellows, faculty and other scientific staff from RCB, and THSTI. The mini-symposium included seminars by scientists from AIST and RCB, with a focus on molecular mechanisms underlying health and disease, and utilizing imaging for disease diagnostics and therapeutics. Representatives from industry which focus on imaging, biomarkers and biotechnology research also discussed their products and their utility in disease related research. The mini-symposium ended with a brief networking session, where the participants interacted with the speakers and industry representatives.

The 5th Molecular Virology meeting was organized jointly with THSTI on 11th and 12th February, 2017. The meeting covered both basic and applied research and provided a platform to discuss latest developments in areas of host-virus interactions, immune responses and vaccine development, molecular biology, evolution and epidemiology of viruses and emerging therapeutics and diagnostics. The meeting brought together leading researchers working on different aspects of Virology and included poster presentations by the students from various institutes.

Lectures delivered/ Conferences attended/ Visits abroad

Dr. Saikat Bhattacharjee

1. Delivered an invited lecture titled 'Plant immune modulators and their strategic coupling to signaling pathways during effector-triggered immunity', at the Interactive Meet on Molecular Intricacies of Plant Associated Microorganisms (MIPAM), organized at Indira Gandhi National Tribal University (IGNTU), Amarkantak, MP, October 27–29, 2017.
2. Delivered an invited lecture titled 'Effector-triggered Immunity in Plants: Strategic deployment of immune modulators mediate robust coupling to defense signaling pathways', at the 2016 Lipid Meeting organized at Amity University, Manesar, December 14-15, 2016.

Dr. Divya Chandran

1. Delivered an invited lecture titled 'Genome-wide miRNA:mRNA integrome analysis of compatible and incompatible legume-powdery mildew interactions', at the National Conference of Plant Physiology, organized by the University of Agricultural Sciences, Bengaluru, 8-10 December 2016.
2. Delivered invited lecture titled 'Dual RNASeq analysis of legume-powdery mildew interactions' at the Genomics Analysis and Technology Conference, Mumbai, 10-11 February 2017.
3. Delivered invited lecture titled 'Genomics-enabled insights into legume-powdery mildew interactions' at the Genomics Analysis and Technology Conference, Bhubaneswar, 8-9 September 2017.
4. Delivered invited lecture 'Functional genomics approaches to unravel plant-pathogen interactions in legumes' at the "Plant-Microbe Interactions in Plant Health, Disease and Biocontrol UGC-DRS Seminar", organized at Sayajirao University, Baroda on 4 October 2017.
5. Attended the conference titled 'Structural Proteomics of Macromolecular Complexes using X-ray crystallography and Mass-spectrometry' held at the Regional Centre for Biotechnology, Faridabad during 18-20 December 2016.

Dr. Prasenjit Guchhait

1. Delivered an invited lecture titled 'Altered immune responses in hemolytic disorders' at the '7th Symposium on Frontiers in Molecular Medicine', organized by JNU, New Delhi, March 2017.

Dr. Deepti Jain

1. Delivered a science talk titled 'Transcription regulation of gene expression' at the event announcing India-ESRF partnership at Prithvi Bhavan, Ministry of Earth Sciences, Lodhi Road, New Delhi, June 19, 2017.
2. Delivered an invited talk titled 'Excitement of Discoveries in Science – Pursuing a career in Research' at Department of Microbiology, Bhaskaracharya College of Applied Sciences (University of Delhi) on January 27, 2017.
3. Delivered an invited lecture titled 'Transcription regulation of flagellar gene network in Pseudomonas aeruginosa' at the 2nd International Conference on Regulatory network architecture in bacteria, organized by the SASTRA University, Tanjavur, December 16-18, 2016.
4. Delivered an invited lecture titled 'ATP induced structural remodelling in the antiactivator FleN enables formation of the functional dimeric form' at the 44th-National Seminar on Crystallography, organized by IISER-Pune, July 10-13, 2016.

Dr. Pinky Kain

1. Attended the 'Annual Fellows Meeting', Hyderabad by Wellcome Trust/DBT India Alliance, May 18-20, 2017.

Dr. Vengadesan Krishnan

1. Delivered an invited lecture titled 'Structural basis of pilus assembly in Probiotic Lactobacillus rhamnosus GG' at the 45th National Seminar on Crystallography (NSC45), organized by the Indian Institute of Technology (BHU), Varanasi, July 9-12, 2017.
2. Delivered an invited lecture titled 'Visualization of bacterial pili' in the 1st DAILAB@RCB Symposium (PIKNIKH series XV), Cellular Mechanism in Health and Disease-Building on Bioimaging & Beyond the Borders held at RCB, Faridabad on February 2, 2017.
3. Delivered an invited lecture titled 'Structural insights into pilus formation in probiotic Lactobacillus rhamnosus GG' at the International summit on Probiotics Health and Nutraceuticals (IPN-2016), Baltimore, USA, September 7-9, 2016.
4. Participated and member of organizing/advisory committee in 'National Workshop on Strengthening Open Access (OA) Initiatives in India' at NBRC, Manesar, June 23, 2017.
5. Coordinated workshop on Protein Crystallization titled 'Structural Proteomics of Macromolecular Complexes using X-ray crystallography and Mass-spectrometry', at RCB, Faridabad, December 18-20, 2016.
6. Attended DeLCON Meeting held at ICGEB, New Delhi, December 19, 2016.
7. Attended 29th DeLCON Nodal Officer's Meeting held at RGCB, Thiruvananthapuram, September 2, 2016.
8. Visited MX beamlines at European Synchrotron Radiation Facility (ESRF), France, May 11-16, 2017 under DBT-ESRF partnership programme.

Dr. Megha Kumar

1. Attended the '18th International Congress of Developmental Biology (ISDB) Meeting' 2017, Singapore and presented a poster entitled 'Role of Dynein Light Intermediate Chains in Embryonic divisions and Vertebrate Embryogenesis'.

Dr. Tushar Kanti Maiti

1. Delivered an invited lecture titled 'Chemical crosslinking mass spectrometry in protein structure and molecular interactions' at the education day program at the '8th Annual Meeting of the Proteomics Society, India, 3rd Meeting of Asia Oceania Agricultural Proteomics organization and International Conference on Functional and Interaction proteomics: Application in Food and Health', held at New Delhi, December 14-17, 2016.
2. Delivered an invited lecture titled 'Nitric oxide, a critical gasotransmitter regulating Parkinson's disease pathology' at the International Conference on Molecular Signaling: Basics to Applications (ICMS-2017), organized at AU-KBC Research Centre, Anna University, Chennai, India, January 10-12, 2017.

Dr. Sam J. Mathew

1. Delivered a talk titled 'Myosin Heavy Chain-Embryonic is a Novel Regulator of Skeletal Muscle Differentiation' at the 1st RCB-AIST mini-symposium held at RCB, Faridabad, February 2, 2017.
2. Delivered an invited lecture titled 'Myosin Heavy Chain-Embryonic is a Key Regulator of Skeletal Muscle Differentiation During Mammalian Embryonic and Fetal Development' at the Biennial Indian Society for Developmental Biology (InSDB) conference, IISER Pune, June 21-24, 2017.
3. Organized the 1st RCB-AIST mini-symposium on 'Cellular Mechanisms in Health and Disease' held at RCB, Faridabad, February 2, 2017.
4. Attended the '7th Annual Fellows Meeting' organized by the Wellcome Trust-DBT India Alliance, Hyderabad, May 18-20, 2017 and presented a poster entitled 'Myosin Heavy Chain-Embryonic is a Key Regulator of Skeletal Muscle Differentiation During Mammalian Embryonic and Fetal Development'.
5. Visited the Department of Human Genetics, University of Utah, USA, as part of the "work outside the host institution" scheme of the "Wellcome Trust-DBT India Alliance Intermediate Fellowship" awarded for the project entitled "The role of developmental myosin heavy chains in skeletal muscle development, regeneration, homeostasis and disease", during 17/12/2016 to 23/01/2017 and 24/05/2017 to 22/06/2017.

Dr. Sivaram V. S. Mylavarapu

1. Delivered an invited lecture titled 'Motoring through Mitosis' at the 2nd National Conference on Understanding the Mechanism and Challenges of Complex Diseases (UMCCD-2), organized by Shaheed Rajguru College of Applied Sciences for Women, University of Delhi, January 24-25, 2017.
2. Delivered invited lecture titled 'Motoring through Mitosis' at the symposium on New Directions in Cell Signaling, organized by Amity University Haryana, April 21, 2017.
3. Attended the '3rd international Meeting on Chromosome Stability' at IISER, Thiruvananthapuram, December 15-18, 2016.
4. Attended the '5th Molecular Virology Meeting' at the Translational Health Science and Technology Institute, Faridabad from February 11-12, 2017.

Dr. Deepak T. Nair

1. Delivered an invited lecture titled 'DNA Polymerase IV, reactive oxygen species and antibiotics: A lethal combination' at Indraprastha Institute of Information Technology, New Delhi, September 5, 2017.
2. Delivered an invited lecture titled 'Mechanism of toroid formation around DNA by Mismatch Sensor Protein' at the 5th Annual Science Festival titled 'BioSparks' organized by School of Life Sciences, Jawaharlal Nehru University, Delhi, March 30, 2017.
3. Delivered an invited lecture titled 'Reactive oxygen species play an important role in the bactericidal activity of quinolone antibiotics' at the 19th International Conference on Emerging Infectious Diseases, organized by the United States-Japan Cooperative Medical program of the National Institutes of Health, USA and held in Seoul (South Korea), February 7-10, 2017.
4. Delivered an invited lecture titled 'Ying and Yang: The conflicting roles of DNA polymerase IV in oxidative stress' at 'Structural Proteomics of Macromolecular complexes using X-ray crystallography & Proteomics' meeting held at Regional Centre for Biotechnology, Faridabad, December 18-20, 2016.
5. Attended the 'BioSparks' Meeting, held at School of Life Sciences, Jawaharlal Nehru University, New Delhi, March 30, 2017.
6. Attended the '19th International Conference on Emerging infectious diseases (EID)', Seoul, February 7-10, 2017.
7. Attended workshop on 'Structural Proteomics of Macromolecular complexes using X-ray crystallography & Proteomics' at the Regional Centre for Biotechnology, Faridabad, December18-20, 2016.
8. Attended 'Guha Research Conference' 2016, Diu, December 8-12, 2016.
9. Visited Seoul (South Korea) to deliver invited lecture at the '19th International Conference on Emerging Infectious Diseases (EID)' during February 7-10, 2017.
10. Visited Grenoble (France) for training in data collection at Structural Biology Beamlines of the European Synchrotron Radiation Facility, during March 15-17, 2017.
11. Visited Grenoble (France) to collect data from macromolecular crystals at the European Synchrotron Radiation Facility, during July 22-25, 2017.

Dr. Sudhanshu Vrati

1. Delivered an invited lecture titled 'Development of a rotavirus vaccine: the India story' at GADVASU, Ludhiana, November 17, 2016

Academic Activities Lectures delivered/ Conferences attended/ Visits abroad

2. Delivered opening lecture titled 'Development of a rotavirus vaccine: the India story' at IIT Kharagpur, November, 25-26 2016.
3. Delivered Inaugural Lecture at 5th Molecular Virology Meeting titled 'Role of host cell proteins in Japanese encephalitis virus replication' at RCB, Faridabad, 11-12 February 2017.
4. Delivered inaugural day lecture titled 'Development of a rotavirus vaccine: the India story' at AMU, Aligarh, March 16, 2017.
5. Delivered inaugural day lecture titled 'Development of a rotavirus vaccine: the India story' in Manipal Research colloquium, organized by Manipal University, April 4, 2017.
6. Delivered keynote lecture 'Development of a rotavirus vaccine: the India story' at IVRI, Mukteswar, June 10, 2017.
7. Delivered an invited lecture 'Role of host cell proteins in Japanese encephalitis virus replication' at South Asian University, New Delhi, September 13, 2017.

Memberships of Professional/ Academic bodies/ Editorial boards/ Review boards

Dr. Saikat Bhattacharjee
1. Review editor, Frontiers in Plant Science

Dr. Divya Chandran
1. Review Editor, Frontiers in Plant Science: Plant Biotic Interactions, Frontiers Publishing Group

Dr. Prasenjit Guchhait
1. Member, Editorial Board, Annals of Clinical and Experimental Immunology
2. Member, Editorial Board, Austin Hematology
3. Member, Editorial Board, Cardiology: Open Access

Dr. Deepti Jain
1. Member, Indian Crystallography Association
2. Member, Society of Biological Chemists

Dr. Vengadesan Krishnan
1. Indian Crystallographic Association (ICA)
2. Indian Biophysical Society (IBS)
3. International Union of Crystallography (IUCr)

Dr. Megha Kumar
1. Member, Indian Society of Developmental Biology
2. Member, American Association of Anatomists, USA
3. Member, Society of Developmental Biology SDB, USA
4. Member, American Sign Language Association (ASL), Clemson University chapter, USA
5. Member, Bombay Natural History Society, India

Dr. Tushar Kanti Maiti
1. Member, Proteomics Society of India
2. Member, Editorial Board of Scientific Reports, Nature Publishing Group
3. Member, Editorial Board of Frontiers in Chemistry

Dr. Sam J. Mathew
1. Member, Indian Society for Developmental Biology (InSDB)

Dr. Sivaram V. S. Mylavarapu
1. Life member, Indian Society for Cell Biology
2. Life member, Society of Biological Chemists, India

Dr. Deepak T. Nair
1. Member, Guha Research Conference
2. Member, Indian Crystallography Association
3. Member, Society of Biological Chemists
4. Member, Board of Studies, Regional Centre for Biotechnology
5. Invited Member of the Biochemistry, Biophysics, Microbiology & Molecular Biology Program Advisory Committee of the Science & Engineering Research Board.
6. Member of the Expert Committee to review Indo-Japan collaborative projects of the Department of Biotechnology.

Dr. Sudhanshu Vrati
1. Life Member, Society of Biological Chemists (India)
2. Life Member, Association of Microbiologists of India
3. Life Member, Indian Society of Cell Biology

Distinctions, Honours and Awards

Dr. Prasenjit Guchhait

1. Ad-Hoc reviewer for Journals: Plos One, Journal of Immigrant and Minority Health, Scientific Reports, Thrombosis Research (2016-2017).

Dr. Deepti Jain

1. SERB Young Investigator Award, Department of Science and Technology, Government of India

Dr. Megha Kumar

1. DST-INSPIRE Faculty award (2016)

Dr. Sam J. Mathew

1. Chaired Session of scientific presentations at the '7th Annual Fellows Meeting' organized by the Wellcome Trust-DBT India Alliance, Hyderabad, May 18-20, 2017.
2. Fellowship supervisor of Dr. Masum Saini, who has been recommended for a Wellcome Trust-DBT India Alliance Early Career Fellowship in June 2017.
3. Invited to participate as a mentor at the Wellcome Trust-DBT India Alliance Science Communication workshop, New Delhi, 7-8 September, 2017.

Dr. Deepak T. Nair

1. "Shanti Swarup Bhatnagar Prize" in Biological Sciences (2017) awarded by Council of Scientific and Industrial Research (Government of India).

Dr. Sudhanshu Vrati

1. Fellow: Indian Academy of Sciences
2. Fellow: National Academy of Sciences, India
3. Elected Member: Guha Research Conference
4. Independent Director: BIBCOL, Bulandshahar

Publications

Original peer-reviewed articles

1. Kidwai S, Park C, Mawatwal S, Tiwari P, Jung MG, Gossain T, Kumar P, Alland D, Kumar S, **Bajaj A**, Hwang Y, Song CS, Dhiman R, Lee Y and Singh R (2017). The dual mechanism of action of 5-Nitro-1,10-Phenanthroline against Mycobacterium tuberculosis. Antimicrobial Agents & Chemotherapy (Accepted).
2. Hussain T, Saha D, Purohit G, Kar A, Mukherjee AK, Sharma S, Sengupta S, Dhapola P, Maji B, Vedagopuram S, Horikoshi NT, Horikoshi N, Pandita RK, Bhattacharya S, **Bajaj A**, Riou JF, TK Pandita and Chowdhury S (2017). Transcriptional control of CDKN1A (p21/CIP1/WAF1) by TRF2 through the REST repressor complex. Scientific Reports (Accepted).
3. Sreekanth V, Medatwal N, Pal S, Kumar S, Sengupta S and **Bajaj A*** (2017). Molecular self-assembly of bile acid-phospholipids controls the delivery of doxorubicin and mice survivability. Molecular Pharmaceuticals, 14: 2649.
4. Yavvari P, Gupta S, Arora D, Nandicoori V, Srivastava A and **Bajaj A*** (2017). Clathrin independent killing of intracellular mycobacteria and biofilm disruptions using synthetic antimicrobial polymers. Biomacromolecules 18: 2024.
5. Meteignier L, Zhou J, Cohen M, **Bhattacharjee S**, Brosseau C, Caamal Chan M, Robatzek S and Moffett P (2016). NB-LRR signaling induces translational repression of viral transcripts and the formation of RNA processing bodies through mechanisms differing from those activated by UV stress and RNAi. Journal of Experimental Botany, 67(8): 2353-2366.
6. Ojha A, Nandi D, Batra H, Singhal R, Annarapu GK, Bhattacharyya S, Seth T, Dar L, Medigeshi GR, Vrati S, Vikram NK and **Guchhait P*** (2017). Platelet activation determines the severity of thrombocytopenia in dengue infection. Scientific Reports, 7: 41697.
7. Tashi T, Reading NS, Moore LG, Wuren T, Hu H, Teng F, Shestakova A, Lorenzo F, Burjanivova T, Koul P, **Guchhait P**, Wittwer C, Julian CG, Shah B, Huff CD, Gordeuk VR, Prchal JT* and Ge R* (2017). Gain-of-Function EGLN1 Prolyl Hydroxylase (PHD2 D4E:C127S) in combination with EPAS1 (HIF2) polymorphism lowers hemoglobin concentration in Tibetans. Journal of Molecular Medicine 95(6): 665-670.
8. Singhal R, Chawla S, Rathore D, Bhasym A, Annarapu GK, Sharma V, Seth T and **Guchhait P*** (2016). Development of pro-inflammatory phenotype in monocytes after engulfing Hb-activated platelets in hemolytic disorders. Clinical Immunology, 175: 133-142.
9. Annarapu GK, Singhal R, Gupta A, Chawla S, Batra H, Seth T, and **Guchhait P*** (2016). HbS binding to GP1b activates platelets in sickle cell disease. PLoS One, 11(12): e0167899. doi:10.1371/journal.
10. Chanchal, Banerjee P and **Jain D*** (2017). ATP induced structural remodeling in the antiactivator FleN enables formation of the functional dimeric form. Structure, 25: 243.
11. Harshita, Chanchal and **Jain D*** (2016). Cloning, expression, purification, crystallization and initial crystallographic analysis of FleN from Pseudomonas aeruginosa. Acta Crystallographica. F72:135.
12. Mishra AK, Megta AK, Palva A, von Ossowski I and **Krishnan V*** (2017). Crystallization and X-ray Crystallographic Analysis of SpaE, a basal pilus protein from the gut-adapted Lactobacillus rhamnosus GG. Acta Crystallographica Section F: Structural Biology Communications, 73: 321.
13. Hanpuda P, Bhattacharya S, Singh AK and **Maiti TK*** (2017). Ubiquitin Recognition of BAP1: Understanding its Enzymatic Function. Bioscience Reports (Accepted).
14. Kumar S, Jangir DK, Kumar R, Kumari M, Bhavesh NS and **Maiti TK*** (2017). Role of sporadic Parkinson disease associated mutations A18T and A29S in enhanced alpha synuclein fibrillation and cytotoxicity. ACS Chemical Neuroscience DOI: 10.1021/acschemneuro.6b00430.
15. Kumar R, Jangir DK, Verma G, Shekhar S, Hanpude P, Kumar S, Kumari R, Bhavesh NS, Jana NR and **Maiti TK*** (2017). S-nitrosylation of_UCHL1_induces its structural instability and promotes -synuclein aggregation. Scientific Reports, 7: 44558.
16. Mukherjee S, Mukherjee S, **Maiti TK**, Bhattacharya S and Sinha Babu SP (2017). A novel ligand of Toll-like receptor 4 from the sheath of Wuchereria bancrofti microfilaria induces proinflammatory response in macrophages. The Journal of Infectious Diseases, 215(6): 954.

17. Mukherjee S, Chattopadhyay M, Bhattacharya S, Dasgupta S, Hussain S, Bharadwaj SK, Talukdar D, Usmani A, Pradhan BS, Majumdar SS, Chattopadhyay P, Mukhopadhyay S, **Maiti TK**, Chaudhuri MK and Bhattacharya S (2017). A Small Insulinomimetic Molecule Also Improves Insulin Sensitivity in Diabetic Mice. PLoS One, 12: e0169809.

18. Mahale SP#, Kumar M#, Sharma A#, Babu A, Ranjan S, Sachidanandan C and **Mylavarapu SVS*** (2016). The Light Intermediate Chain 2 Subpopulation of Dynein Regulates Mitotic Spindle Orientation. Scientific Reports, 6(1): 22, doi: 10.1038/s41598-016-0030-3.

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Academic Activities

Distinguished Lectures

Date	Speaker	Title
October 05, 2017	Prof. Seyed E. Hasnain, Jamia Hamdard, New Delhi	Understanding the Making of TB to Find Ways to Unmake it
February 6, 2017	Prof. Walter Gassmann Division of Plant Sciences, University of Missouri, Columbia, USA	Staying in Touch: Functions of the Arabidopsis Immune Adaptor Protein SRFR1
December 19, 2016	Prof. Arshad B. Desai Ludwig Cancer Research and the University of California, San Diego	How Cells Get the Right Genome

Scientific Colloquia

Date	Speaker	Title
October 05, 2017	Prof. Vidita Vaidya TIFR, Mumbai	Role of serotonin in the programming of psychiatric vulnerability
October 05, 2017	Dr. Rashna Bhandari CDFD, Hyderabad	Protein pyrophosphorylation - ten years and counting
October 05, 2017	Dr. Debashis Mitra NCCS, Pune	Cellular factors and signaling pathways: novel targets in the fight against HIV/AIDS

Extramural
Activities

Extramural Activities

Dr. Saikat Bhattacharjee

External advisor for PhD student at AcSIR, Ghaziabad (June, 2017).

External Doctoral Committee member for a PhD student at THSTI, Faridabad (June, 2017).

External member in interview committee for selection of PhD candidates at CSIR-IGIB, Delhi (July, 2017).

Mentoring a MSc student from Punjab Agricultural University, Ludhiana as a part of IASc-INSA-NASI Summer Research Fellowship Programme (July-August, 2017).

Mentoring a DST-INSPIRE fellow and Fulbright-Nehru Visiting Scholar, University of Hyderabad (July-September, 2017).

Participated in Centenary Celebrations and served as a poster judge at the Bose Institute, Kolkata, 8th - 10th February, 2017.

Ranked 1st in SGRF Genome Project Grant 2016 (to Dr. Jewel Jameeta Noor). Project title: Selective mRNA export: a cellular switch to initiate and regulate plant innate immunity to bacterial pathogens (Genome grant Application No. 141). This would facilitate RNAseq of 6 samples.

Dr. Deepti Jain

Convenor of the International Symposium cum workshop on "Structural Proteomics of Macromolecular Complexes using X-ray crystallography and Mass-spectrometry", organized at RCB, Faridabad, December 18-20, 2016.

Dr. Pinky Kain

Organized "Science outreach program" at Sarvodaya Vidyalaya, INA colony, Delhi, July 7, 2017.

Dr. Sivaram V. S. Mylavarapu

External doctoral committee member for multiple PhD students at IGIB, New Delhi for promotion from Junior Research Fellow to Senior Research Fellow.

External doctoral committee member for multiple PhD students at THSTI, Faridabad.

Dr. Deepak T. Nair

Coordinator of the European Synchrotron Access program of the Regional Centre for Biotechnology. The program provides access to high intensity synchrotron radiation to scientists from India and the Regional Centre for Biotechnology runs this program on behalf of the Department of Biotechnology.

Coordinator of the project titled "Big Data Initiatives in Astronomy and Biology" a collaborative project between the Regional Centre for Biotechnology and the Inter University Centre for Astronomy and Astrophysics.

International and National Networking

RCB-DAILAB

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

A DBT-AIST joint lab (DAILAB) for advanced research training in bio-imaging and biotechnology, including high end in vivo and in vitro imaging, has been established at RCB. The DAILAB will facilitate joint research collaborations engaging Indian and Japanese scientists and support selected Indian researchers for training in specialized areas of bio-imaging and biotechnology, both in Japan and in India. The DAILAB at RCB has been set up for training and research in advanced imaging, with in vivo imaging, high end confocal, fluorescent, and bright field imaging, as well as cell culture capabilities. Workshops with a focus on imaging related technologies are planned as part of the DAILAB at RCB, in order to bring together experts and students for theoretical and hands on imaging sessions, taking advantage of the facilities available as part of this initiative.

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

BM14 Project

An agreement was signed between Department of Biotechnology, European Molecular Biology Laboratory (EMBL) and European Synchrotron Radiation Facility (ESRF) in 2008, which enabled Indian scientists to visit the BM14 beamline located in ESRF to collect X-ray diffraction data on the experimental products developed in their respective labs. This project began operations in the spring of 2009 and the initial sanction was for five years. In 2014, the management of BM14 beamline for the extended period of 2 years was transferred to RCB. To facilitate this extension and effective management, a tripartite agreement was signed between RCB, EMBL and ESRF. Since its inception, the project has helped many scientists and students from all over India and has resulted in more than 200 publications in leading international journals. The project closed down in 2016 and RCB in partnership with ESRF and with support from DBT has started a new ESRF Access Program (see below).

The India-ESRF partnership

To build on the successful first phase of association with ESRF (2008-2016), the Regional Centre for Biotechnology (RCB), with support from the Department of Biotechnology, made a new and advanced arrangement with the ESRF. The agreement provides access to multiple and much better experimental stations for macromolecular crystallography, small angle X-ray scattering (SAXS) and the cryo-electron microscopy. At a function held in New Delhi on 19.06.2017, Dr. Harsh Vardhan (Minister of Science & Technology, Earth Sciences and Environment, Forests and Climate change, Govt. of India) announced a new partnership between India and the European Synchrotron Radiation Facility (ESRF). To this end an agreement was signed by Prof. Sudhanshu Vrati (Executive Director, Regional Centre for Biotechnology) and Dr. Francesco Sette (Director-General, ESRF) to enable the use of the facility's synchrotron radiation sources for medium-term non-proprietary research. India has thus become the 22nd country to join the ESRF and this association was possible due to the strong support from the Department of Biotechnology (Government of India).

The ESRF, located at Grenoble in France, produces X-rays of high intensity and quality and these X-rays are used to provide high resolution images of natural and artificial matter at the level of nanometers. These high intensity X-rays are particularly useful for macromolecular crystallography, which is used to obtain three-dimensional structures of proteins and other biological macromolecules. These structures are utilized to derive insights regarding the mechanism and functions of these molecules. The derived insights are used to develop a variety of biotechnological applications, including the development of new drugs and vaccines to protect and cure diseases that plague humans and formulation of molecular strategies to protect plants and animals from diseases.

Since the start of this new agreement, scientists from 13 different institutes from all over India have obtained x-ray diffraction and small angle X-ray scattering data for different macromolecules and macromolecular assemblies. The list of institutions are as follows: - All India Institute of Medical Sciences, New Delhi; Central Drug Research Institute, Lucknow; International Centre for Genetic Engineering & Biotechnology, New Delhi; CSIR-Institute of Genomics & Integrative Biology, New Delhi; Indian Institute of Science, Bangalore; Indian Institute of Science Research & Education, Pune; Indian Institute of Technology-Bombay, Mumbai; Institute of Life Sciences, Bhubaneshwar; Institute of Microbial Technology, Chandigarh, School of Life Sciences, Jawaharlal Nehru University, New Delhi; Regional Centre for Biotechnology, Faridabad; University of Madras, Chennai and Institute of Stem Cell & Regenerative Medicine, Bangalore. The access to this international facility will enable Indian scientists to formulate innovative solutions to problems faced by the nation in public health and agriculture.

NCR Biotech Science Cluster, Faridabad

RCB is an integral founding partner of the NCR Biotech Science Cluster (BSC) at Faridabad in the national capital region (NCR) of Delhi. The BSC supports discovery of basic mechanisms in biology and development of novel technologies and reagents associated with biotechnology. The cluster aims to facilitate public-private

partnerships for the development of biotechnology business incubators and technology parks, including creative partnerships with Biotech & Pharma entrepreneurs and distributors. The cluster will network with the potential constituent institutions to create a synergistic ecosystem for accelerating discoveries and facilitating translation of these discoveries to real world solutions in the field of health care and agriculture and their commercialization. In terms of infrastructure, the cluster will have a state -of-the - art technology platform center, animal facilities and bio safety containment laboratories.

Advanced Technology Platform Centre (ATPC)

The primary goal of the Advanced Technology Platform Centre is to accelerate innovation in biology and biotechnology and thus contribute towards improving the Indian economy. The Centre will plug a huge gap in the innovation pipeline that has previously attenuated the ability of Indian researchers to realize their true potential. Towards this end, the ATPC will house cutting edge technologies to enable researchers within all the constituent partner institutes to conduct experiments that will provide deep insight in biological processes and provide the best opportunity to translate these discoveries for commercialization. At present, three facilities are operational in ATPC and these include the Protein Purification Facility, the Flow Cytometry facility and the Mass Spectrometry Facility. Facilities such as Optical Microscopy, Electron Microscopy, Mass Spectrometry, Genomics, Molecular Interactions and facility to enable Animal Experiments will be developed in the ATPC in the near future. Access to these cutting edge facilities will help Indian researchers find new solutions to problems faced by the nation in public health, agriculture and skill development.

NCR Biotechnology Business Incubator

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

Technology Advancement Unit (TAU)

The Indo-Swiss Collaboration in Biotechnology (ISCB) is a bilateral research and development programme, jointly funded and steered by Swiss Agency for Development & Cooperation (SDC) and Department of Biotechnology (DBT). SDC has established Project Monitoring Unit (PMU) in Switzerland to facilitate and monitor the projects carried out under ISCB. DBT has also sanctioned the project "Establishment of Technology Advancement Unit (TAU)" on 9.9.2014 for 5 years at Regional Centre for Biotechnology (RCB) as an adjunct to the Project Management Unit (PMU) of SDC.

The mandate of ISCB is to develop products and biotechnological processes which can be used or applied for the benefit of the rural committees in India and to build capacities of Indian Institutes and promote partnerships between Swiss and Indian Institutes as well as private companies. After an external evaluation in 1999, the new ISCB programme was commissioned. The new programme focused on innovative agricultural and environmental (bio) technologies. Networks with at least one Swiss and one Indian project partner are supported. The focus area of Phase I-III (1999-2012) were control of stress factors in pulses, molecular breeding and genomics of wheat, biopesticide, biofertilizers, bioremediation and biosensors. Additionally, in Phase IV, the participatory values and approach and socio- economic component was introduced.

Phase IV (15) of ISCB (January 2013- October 2017)

The details of 4 network projects being implemented during Phase IV (15) and their progress & technology advancement are as follows:

Network (Duration & Cost)	Partners	Description	Advancement to next development stage
BIOFI Network (3 years from 21.10.2014 at total cost of Rs. 358.524 lakh)	Bharathiar University, Coimbatore, Pondicherry University, UAS, Bangalore, ICRISAT, Hyderabad, MS. Swaminathan Research Foundation, Chennai, University of Basel, Research Institute of Organic Agriculture, Ackerstrasse, Bern University of Applied Sciences (HAFL) Langgasse	Biofertilization and "bioirrigation" for sustainable mixed cropping of pigeon pea and finger millet (BIOFI)	Field trials already on-going along with Socio-economic partners (eco-enterprise) Compliance at various stages
Indo-Swiss Cassava Network (3 years from 20.10.2014 at total cost of Rs. 1.50 Crores)	Central Tuber Crops Research Institute, Thiruvananthapuram, Tamil Nadu Agriculture University, Coimbatore, University of Basel	Development of virus (CMD) and white fly resistant Cassava.	Facilitation in biological material transfers - to build seed distribution / replacement systems in cassava in a multiparty approach - disseminate technology and ensure extension through AICRPTC.
Ragi Network (3 years from 23.01.2015 at total cost of Rs. 3.49 Crores)	University of Agricultural Sciences, Bangalore, National Academy of Agricultural Research Management (NAARM), Hyderabad, ETH Zurich, University of Zurich (UZH), Functional Genomics Centre, Zurich (FGCZ)	Genetic enhancement & bioavailability – Finger millet (Ragi)	Facilitation in biological material transfers -GWAS studies, Validated QTLs for selected shortlisted traits, Advancing few lines from already characterized germplasm through All India Coordinated Millet Improvement Program
Pigeon Pea Network (3 years from 04.08.2015 at total cost of Rs. 6.118 Crores)	National Research Centre on Plant Biotechnology, New Delhi, Indian Agricultural Research Institute, New Delhi, National Bureau of Plant Genetic Resources, New Delhi, ETH & UZH Functional Genomics Centre, Zurich, Bern University of Applied Sciences (HAFL) Langgasse	Improvement of Pigeon pea for plant type, pod borer resistance and moisture stress tolerance	Facilitation of intellectual property protection of developed Micro-chip -Number of lines in internal evaluation and advance shortlisted entries for AICRP trials

Anticipated Outcome

The four networks, each consisting of biotechnologists, plant breeders and socio-economists in a constructive collaboration are expected to achieve the following:

- BIOFI (biofertilizer/ bioirrigation) network: Development of an innovative package of biofertilization and bioirrigation in a specified intercropping system of pigeon pea and finger millet.
- Cassava network: Capacity building of Indian scientists in cassava biotechnology, identification and evaluation of non-transgenic varieties resistant of cassava mosaic virus.
- Ragi (finger millet) network: Identification of the genetic resources for targeted finger millet improvement and development of genomic tools for molecular breeding.
- Pigeon Pea network: Development of an advanced breeding line of pigeon pea with dwarf stature, early maturity and high yield and advanced research towards pest resistance (pod borers).

Outcome 1

- Biofertilizer Network under ISCB programme that conducted both basic and translational research in order to develop sustainable biofertilizer technologies to facilitate product delivery, in partnership with the industries, to small and marginal farmers, leading to poverty alleviation. The network project has resulted in development of novel technologies for increasing crop productivity through application of effective bio-inoculants (bio-fertilizers). TAU has been involved in the technology transfer and advancement of biofertilizer technology to private industries, on behalf of technology developers. The effectiveness of this technology has been successfully demonstrated in wheat (Triticum aestivum) and is presently in validation and product development phase by Nagarjuna Fertilizers and Chemicals Ltd. (NFCL), Hyderabad. The first commercial sale of the product is expected by year 2018.

- Researchers at Assam Agricultural University (AAU), Jorhat in collaboration with University of Basel, have developed marker free transgenic plants expressing Bt genes (Cry1Ac and Cry2Aa), which provide resistance to pod borer infestation. The technology uses a twin binary vector for transformation and the transgenic plants expressing the Bt genes show enhanced tolerance to pod borer (Helicoverpa armigera) and other lepidopteran pests. The effectiveness of Bt in controlling Helicoverpa armigera infestation has already been demonstrated widely in cotton and corn globally including India. The Bt-technology transfer and advancement (through regular monitoring and evaluation) to one private industry Sungro seeds and two public institutions [Punjab Agriculture University (PAU), Ludhiana and Indian Institute of Pulse Research (IIPR), Kanpur] has been facilitated by TAU. BRL1 (Biosafety research level 1) trials in chickpea are undergoing by Sungro and introgression breeding of trait into elite germplasms is undergoing at public institutes (PAU and IIPR).

Outcome 2

Outcome 2 was strongly built on the assumption that the projects of the networks will be marketable, however, this cannot be achieved in the short time remaining in the program.

Outcome 3

TAU has assisted DBT in facilitating financial release and review of progress reports of network projects. TAU also facilitated project partners and the participating institutions in NBA Notification, Biological Material Transfer (to and from India to Switzerland), IP Management and Capacity building.

Phase V (16) of ISCB (November 2017- October 2019)

SDC has taken the decision to end their support to ISCB & phase out the partnership to foster capacity building in a final 2-year phase (Phase V (16) until October 2019. By the end of the final two years (Phase V (16) until October 2019), the 4 network projects, each consisting of biotechnologists, plant breeders and socio-economists in a constructive collaboration are expected to achieve the following:

- In the BIOFI (biofertilizer) network, the concept of bio-irrigation should be tested and proven in an experimental field.

- The focus of the Indo Swiss Cassava network is to advance the non-transgenic cassava line, develop a seed system for hybrids and finalize the capacity building through workshop(s) with stakeholders.

- Both the Ragi and Pigeon Pea networks are expected to deliver valuable basic research data by the end of Phase V.

Biosafety Support Unit (BSU)

Indian Biotechnology Regulations implemented under Rules, 1989 of the Environment (Protection) Act, 1986, are dynamic and complementing with emerging technologies used in the research, product development and progress in science of risk assessment. Department of Biotechnology, Government of India has undertaken several reforms in biotechnology regulatory system including "Establishment of Biosafety Support Unit (BSU)", with trained and skilled scientists with specialization in various scientific disciplines, in partnership with Regional Centre for Biotechnology (RCB). The mandate of BSU includes (a) assisting RCGM/GEAC in scrutiny of applications received for risk assessment and preparing reports to facilitate decision making, (b) developing guidelines and protocols to assist researchers and industries for generating biosafety data to address the challenges raised by the emerging areas of biotechnology and, (c) providing desired scientific information on emerging biosafety issues.

BSU has two risk assessment groups i.e. Agriculture and Bio-Pharma. At present these groups are headed by Chief (Agriculture and Bio-Pharma), under overall supervision of Chief Scientific Officer (CSO) & comprise of a team of ten scientists having specialization in the field of Plant Biotechnology, Plant Protection, Microbiology, Environmental Sciences, Biochemistry, Genetics and Breeding, Bioprocessing Engineering, Bioanalysis, Toxicology and Veterinary Sciences.

Major activities undertaken by BSU during the year 2017 include:

- Provided assistance to RCGM/GEAC (Statutory bodies established under Rules 1989 of Environment Protection Act, 1986) in the scrutiny of the applications received for conducting research in biotechnology and monitoring field trials. The activities of BSU includes desk review of all applications to ensure the completeness of the data requirements, compliance of the approved protocols/procedures to be followed at the time of field trials (Event selection, BRL-I and BRL-II) and preclinical toxicology (PCT) data and other regulatory compliance.

- Assisted the RCGM secretariat in developing revised guidelines and protocols for generating biosafety data to address the challenges raised by the emerging new areas of Biotechnology.

- BSU team is also involved in analyzing the training needs of the personnel engaged in Biosafety regulations and working towards training and capacity building of the personnel in regulatory science.

- BSU is fully engaged in bringing out a new scientific journal "Journal of Biosafety Regulation" in order to provide a platform for scientific community and other stakeholders to publish various aspects of regulatory science.

- BSU provided all necessary requisite services to RCGM/GEAC and assisted RCGM secretariat in organizing scheduled regular meetings of the RCGM, various sub-committees and monitoring teams, etc.

Major accomplishments

1. RCGM/GEAC Related Activities

BSU evaluated 909 applications submitted to RCGM for consideration in thirteen RCGM meetings during the year 2016-17 and extended its support towards conducting the meetings of Review Committee on Genetic Manipulation (RCGM) by preparing Agenda notes and draft recommendations. Further, in-depth desk review was carried out for each of the application/reports submitted by the applicants on confined field trials (CFTs) and pre-clinical trials (PCT). Similarly, applications for import/export/ transfer/receive, and information items in the field of agriculture and pharma were also assessed. Further BSU continued its full support to GEAC by providing Risk assessment and risk management plan (RARMP) documents for each application considered in the GEAC meetings during the year 2016-17. BSU has drafted a comprehensive safety assessment document referred as "Assessment of Food and Environmental Safety (AFES)" document for GE mustard, based on which GEAC has taken its final decision. During this process, BSU analyzed more than 750 comments received during public consultation of GE mustard and prepared the summary report, based on which, GEAC has taken its final decision on GE mustard.

In addition, BSU carried out technical evaluation and provided RARMP documents on specific request of other government institutions for their proposal on Genetically Modified Mosquito, proposal on Wolbachia infected Aedes aegptii and Anopheles mosquito and GE silk worm.

2. Launch of Indian Biosafety Knowledge Portal

BSU has facilitated RCGM secretariat in revising all the application formats that are compatible for online submission to RCGM for import/ export, research, exchange, field trial, preclinical toxicity studies etc. BSU initiated the multi-user functionality testing of the portal before it is hosted on NIC server.

3. Revision and updation of Biosafety Protocols and Guidelines

BSU has undertaken a major activity of revision/ updation of various guidelines related to biosafety of recombinant DNA research. Following guidelines are in the final stages of stakeholder consultation process:

i. Regulations and Guidelines on biosafety of recombinant DNA research and biocontainment

ii. Safety assessment of GE crops containing stacked events

iii. Assessment of GE microbes for environmental release and soil microbes for GE crops

iv. Guidelines on quality evaluation of live recombinant viral vectored vaccines

In addition, BSU has provided inputs in the revision of "Guidelines on Similar Biologicals", regulatory requirements for marketing authorization in India. A checklist for the quality control of recombinant monoclonal antibody has been prepared. Furthermore, the group has provided in-depth inputs on "Testing policy for therapeutic monoclonal antibodies" that are being prepared by National Institute of Biologicals (NIB). Besides, simplified guidelines for exchange, import and export of GMOs and product(s) thereof were prepared to facilitate researchers and industries for quick and easy exchange of DNA / RNA / vectors / seeds / recombinant products to fasten product development process.

4. Support to Food Safety and Standards Authority of India (FSSAI) scientific panel on GM food

BSU prepared comprehensive documents on the GM food labelling policy, wherein the global scenario was compared and a draft proposal for India was presented. Based on the document and the presentation, the proposal was accepted and now is in the advanced stages of approval. In addition, BSU prepared documents defining the notified/ certified GM food testing laboratories in India, which includes the checklist and requirements of laboratories to be certified as GM food testing labs. Another document was prepared defining the principles of risk assessment for GM foods, wherein best international practices were taken into account.

5. Other activities

i. BSU supported 14th and 15th Standing Committee looking into the commercialization of BGII cotton hybrids under EBAM (Event Based Approval Mechanism). BSU scientist analyzed data of BG-II hybrids and came up with threshold level of important parameters such as protein expression, bioefficacy and yield. Such a meta-analysis facilitated in the decision-making process of the standing committee.

ii. BSU have been also involved in various monitoring teams (CCC)? to ensure compliance during confined field trials/ facility evaluation etc.

iii. BSU has also initiated the activity of providing RARM plan documents to the state governments to facilitate their decision making in granting NOC for conducting the field trials in their respective states.

iv. Support to RCGM/GEAC for drafting affidavits/ replies for various Supreme Court cases and 301st report by Parliament standing committee (GM crops and its impact on environment, safety assessment of GE mustard, GM crops and impact on animals). In addition, BSU has drafted scientific documents related to upcoming new technologies like genome editing, germ line gene therapy, resistance of pink bollworm to Bt cotton etc.

6. Biosafety Journal

BSU has initiated the work towards launching the biosafety journal. Under this activity, a team of experts for the editorial board of the journal have been finalized. The Editorial board is comprising of 10 eminent scientists from various countries like India, France, USA, and Australia. Articles from 20 topics that are relevant to biosafety science have been identified. Efforts are being made to get articles and to publish its first issue during the year 2017-18.

Extramural Funding

S. No.	Investigator	Project	Funding Agency	Grant Amount (Rs.)	Duration
1.	Dr. Avinash Bajaj	Temporal targeting of siRNA therapeutics to the gastrointestinal tract (GIT) using chimeric nanogels	Department of Biotechnology	84.31 lakh	2017-20
2.	Dr. Avinash Bajaj	Development of biocompatible surfaces for ESKAPE pathogens	Department of Biotechnology	41.27 lakh	2017-20
3.	Dr. Avinash Bajaj	Molecular engineering of low molecular weight injectable hydrogels with sustained drug release for cancer therapy	Department of Biotechnology	42.60 lakh	2016-19
4.	Dr. Avinash Bajaj	Engineering of self-assembled lipidated nanoparticles for cancer combination therapy	Department of Science and Technology	47.67 lakh	2016-19
5.	Dr. Avinash Bajaj	Targeting persistent infections and multi-drug resistance in bacterial infections and biofilms using engineered synergistic bile acid amphiphile-drug conjugates	Department of Biotechnology	50.12 lakh	2015-18
6.	Dr. Avinash Bajaj	Investigating the role of BLM helicase as a global tumor suppressor: understanding its regulatory loops and using the knowledge for therapeutic and clinical applications in cancer biology	Department of Biotechnology	29.44 lakh	2015-20
7.	Dr. Saikat Bhattacharjee	Understanding role(s) of post-translational modifications by SUMO in regulation, trigger and execution of effector-triggered immunity in plants	Science & Engineering Research Board -DST	35.00 lakh	2017-20
8.	Dr. Divya Chandran	Deriving gene regulatory networks mediating legume host-powdery mildew pathogen cross-talk during compatible and incompatible interactions	Department of Biotechnology (Innovative Young Biotechnologist Award 2015)	43.16 lakh	2016-19
9.	Dr. Divya Chandran	Identification of novel regulators and nodes of response mediating powdery mildew sporulation on legumes	Science & Engineering Research Board -DST	39.11 lakh	2017-20
10.	Dr. Prasenjit Guchhait	Mechanism of rapid propagation of dengue virus during infection (jointly with AIIMS and THSTI)	Department of Biotechnology	Total grant: 1.00 Cr. Grant for RCB: 46.00 lakh	2017-20

S. No.	Investigator	Project	Funding Agency	Grant Amount (Rs.)	Duration
11.	Dr. Deepti Jain	Biochemical and Structural Characterization of the Single Polypeptide Mitochondrial RNA Polymerase – RpoTm	Science & Engineering Research Board -DST	34.81 lakh	2016-19
12.	Dr. Deepti Jain	Partial support for organizing a workshop on "Structural Proteomics of Macromolecular Complexes using X-ray Crystallography and Mass-Spectrometry", RCB, Faridabad	Science & Engineering Research Board -DST	1.00 lakh	2016
13.	Dr. Pinky Kain	Understanding the taste and its modulation using Drosophila melanogaster as a model system	Wellcome trust DBT Alliance intermediate fellowship	3.50 Cr.	2016-21
14.	Dr. Vengadesan Krishnan	Structural investigations of surface nano scale assembly in a gut bacterium	Department of Biotechnology	70.00 lakh	2014-17
15.	Dr. Megha Kumar	Role of dynein Light Intermediate Chains in embryonic divisions and vertebrate embryogenesis	DST-INSPIRE Faculty Award grant	35.00 lakh	2017-22
16.	Dr. Pushpa Kumari	WT/DBT 1A Early Career Grant	Regional Centre for Biotechnology	144.93 lakh	2014-18
17.	Dr. Tushar Kanti Maiti	Inter-institutional programme for Maternal, Neonatal and Infant Sciences: a translational approach to studying PTB (jointly with THSTI, NIBMG, General Hospital, Gurgaon, and Safdarjung Hospital)	Department of Biotechnology	Total grant: 48.85 Cr.; Grant for RCB: 6.13 Cr	2014-19
18.	Dr. Tushar Kanti Maiti	Targeting ubiquitin proteasome system for the anticancer drug development: A peptoid based inhibitor design, synthesis and evaluation	Department of Biotechnology	24.90 lakh	2015-18
19.	Dr. Tushar Kanti 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, THSTI, Gurgaon Hospital and Safdarjung Hospital)	BIRAC Department of Biotechnology Gates Foundation	Total grant: 134.22 lakh Grant for RCB: 61.34 lakh	2016-17

S. No.	Investigator	Project	Funding Agency	Grant Amount (Rs.)	Duration
20.	Dr. Sam J. Mathew	The role of developmental myosin heavy chains in skeletal muscle development, regeneration, homeostasis and disease	Wellcome Trust-DBT India Alliance Intermediate Fellowship	352.00 lakh	2014-19
21.	Dr. Sam J. Mathew	The role of MET-CBL signaling in Rhabdomyosarcoma	DBT Cancer grant Pilot Project for Young Investigators	24.00 lakh	2015-18
22.	Dr. Sam J. Mathew	The Role of Transducin-like Enhancer of Split 3 (TLE3) in Regulating Myogenesis	Science & Engineering Research Board -DST	60.00 lakh	2017-20
23.	Dr. Sivaram V. S. Mylavarapu	Molecular Basis for Silencing of the Spindle Assembly Checkpoint	Department of Biotechnology	35.00 lakh	2013-16 (ongoing, on a no-cost extension)
24.	Dr. Sivaram V. S. Mylavarapu	Prolyl Isomerization of the Dynein Light Intermediate Chains as a Regulatory Driver for Mitosis	Science & Engineering Research Board -DST	48.00 lakh	2017–20
25.	Dr. Deepak T. Nair	The role of DNA Polymerase IV in ROS mediated lethality: Structure and Mechanism	Department of Biotechnology (as part of the National Bioscience Award for Career Development-2014)	15.00 lakh	2016-19
26.	Dr. Deepak T. Nair	Mechanism of mutagenic & translesion DNA synthesis by a mycobacterial Y-family DNA polymerase	Science & Engineering Research Board-DST	59.34 lakh	2015-18
27.	Dr. Deepak T. Nair	Big Data Initiatives in Biology & Astronomy	National Knowledge Network	150.00 lakh	2016-19
28.	Dr. Deepak T. Nair	Molecular Interactions critical for DNA Mismatch Repair	Science & Engineering Research Board-DST	24.91 lakh	2017-20
29.	Dr. Deepak T. Nair	Access to Macromolecular Crytsallography Beamlines of ESRF, France	Department of Biotechnology	1749.41 lakh	2017-19
30.	Dr. Deepak T. Nair	Effect of N^2-Adducts of Deoxyguanosine on DNA Synthesis by Replicative and Translesion DNA Polymerases	Department of Biotechnology	19.90 lakh	2015-18

Infrastructure

Infrastructure

Laboratory Infrastructure

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

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

Macromolecular Crystallography: This facility has an automated nanodispenser for crystallization experiments, vibration free crystallization incubator, UV and light microscopes, two X-ray generators (sealed tube & Metaljet) with optics, detectors and cryostreams.

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

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

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

High Performance Computing Cluster (HPCC): A high performance computing cluster with 8 nodes and a total of 128 processors is available for research in computational biology.

In collaboration with AIST, Japan, a DAILAB has been set up in RCB with the following equipment accommodated: Confocal microscope, Imaging & plate Reader, In vivo Imager, StereoMicroscope, Fluorescence microscopes (2) and an Electroporation device. The Common Instrument Facilities (CIFs) have a number of instruments for probing molecular interactions. These include Blood Cell Analyzer, 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.

Also, equipment such as a laser scanner for biomolecular imaging, gel documentation units, RT-PCR machine and nanodrop spectrophotometer are also available in the CIFs. In addition, 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 are also available for use by researchers. RCB has built capacity to conduct experiments on animals in the Small Animal Facility of the NCR-BSC and will also contribute extensively to the development of a BSL3 facility in the cluster.

Each spacious laboratory here is shared between Principal Investigator (PIs). The laboratories have work and lab preparation benches, storage furniture, seating space with computers for research members, networked PI cabins along with internet and phone access. All laboratories are equipped for conducting research in their specialized areas. Specialized facilities such as cold room, dark room and X-ray rooms are set up for undertaking specialized experimental research. Teaching laboratories for students of the MSc course will be operationalized in near future.

The centre has facilities for laboratory meetings, interactions, discussions teaching and tutorials. The common facilities of the NCR Biotech Science cluster include the auditorium complex, which has two seminar rooms (each with an occupancy of 150 persons) and one such room is fully available for the centre. The central auditorium is utilized for organizing and conducting institutional meetings, seminars, workshops and conclaves.

Digital Initiatives

The requirement to establish a state-of-art IT network and infrastructure was of paramount importance given the geographical location and the requirement of the campus facility location in the NCR-BSC. LAN and Wi-fi connectivity has been successfully established and provided to all users with the high-speed internet connectivity through the National Knowledge Network.

The Centre has been functioning in conformity with the guidelines of the Government of India with regard to guidelines on IPv6 implementation and has also been an active participant in the Government initiatives of the "Digital India Campaign". The Centre has started procurement through "GeM-Government eMarketplace" portal. Access Control Machines have been installed for provision of authorised access to the high-end laboratory facilities in the campus. A very competent & experienced IT service support team has been put in place and the Centre is also in the process of developing and implementing a highly attractive, user-friendly and dynamic web-site.

RCB's e-Library

The library & e-library facility has been fully established with regular subscription of electronic versions of 1170 scientific journals and has more than 600 books. The access to e-journals provided by the DBT Electronic Library Consortium (DELCON) is also available to all the users of the Centre. This facility shall be enhanced in near future with state of art Information & Communication Technology (ICT) labs. The Centre will also establish a library with modern amenities to enable students to acquire basic knowledge in different areas of biotechnology and conduct advanced research in specialized fields.

Financial Statements

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

INDEPENDENT AUDITOR'S REPORT

We have audited the attached balance sheet of REGIONAL CENTRE FOR BIOTECHNOLOGY, 3rd **Milestone, Faridabad Gurgaon Expressway, Faridabad** as on 31st March, 2017 and its Income and Expenditure Account for the year ended on annexed thereto. These Financial Statements are the responsibility of the centre's management. Our responsibilities express an opinion on these financial statements based on our audit.

1. That the centre's balance sheet, Income & Expenditure Account and Receipt and Payment Account Agreement with the books of accounts.
2. We conducted our audit in accordance with auditing standard generally accepted in India. Those standards require that we plan and perform the audit to obtain reasonable assurance about whether the statements are free of material misstatement. An audit includes examining, on a test basis, evidence supporting the amounts and disclosures in the financial statements. An audit also includes assessing the accounting principles used and significant estimates made by the management, as well as evaluating the overall statement presentation. We believe that our audit provides a reasonable basis for our opinion.
3. Subject to accounting policies and notes to accounts as attached, in our opinion and to the best information and according the explanation give to us, the said accounts give a true and fair view:

(a) In Case of Balance Sheet, of the statement of affairs of the centre as at 31.03.2017 and

(b) In case of Income Tax & expenditure account, of the centre during the period ended on 31st March 2017

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: 27/09/2017

REGIONAL CENTRE FOR BIOTECHNOLOGY
BALANCE SHEET AS AT 31ST MARCH, 2017

Amount (In Rs.)

CORPUS / CAPITAL FUND AND LIABILITIES	Schedule		Current Year		Previous Year
CAPITAL GRANTS FOR INFRASTRUCTURE	1	-	16,66,00,236		18,58,74,826
RESERVES AND SURPLUS	2	-	4,15,012		4,15,012
CURRENT LIABILITIES AND PROVISIONS	3(A)	-	5,66,11,128		15,26,49,340
BIOTECH SCIENCE CLUSTER (BSC)	3(B)	-	2,19,68,73,773		2,11,14,83,006
TOTAL			**2,42,05,00,149**		**2,45,04,22,184**
ASSETS					
FIXED ASSETS	4	-	15,88,23,292		12,87,92,799
FUNDS IN SHORT TERM DEPOSITS	5(B)	-	-		4,28,92,000
CURRENT ASSETS,LOANS ADVANCES ETC.	5(A+C)	-	26,77,84,714		33,68,71,695
BIOTECH SCIENCE CLUSTER (BSC)	5(D)		1,99,38,92,143		1,94,18,65,690
a. Capital Work in progress	4	1,95,19,46,843		1,68,66,73,385	
b. Advance to BSC construction.	5(D ii & iii)	3,11,38,229		24,48,57,581	
c. Funds in short term deposits	5 (D i)	34,00,000		34,00,000	
d. Accrued interest & TDS	5(D iv & v)	74,07,071		69,34,724	
TOTAL			**2,42,05,00,149**		**2,45,04,22,184**
SIGNIFICANT ACCOUNTING POLICIES & NOTES TO ACCOUNTS					

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

BIJU MATHEW
SENIOR MANAGER (A&F)

SUDHANSHU VRATI
EXECUTIVE DIRECTOR

RASHMI GUPTA
PARTNER

PLACE: Faridabad
Date 27/09/2017

REGIONAL CENTRE FOR BIOTECHNOLOGY

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31st MARCH, 2017

Amount (In Rs.)

INCOME	**Schedule**	**Current Year**	**Previous Year**
Grants/Subsides	6	14,21,77,971	14,99,22,311
Fees/Subcrpitions	7(i & iii)	3,63,020	2,73,920
Interest on investments on fixed deposits/savings a/c	7(ii)	46,34,401	36,26,788
Deferred Income-Fixed Assets	1	6,92,73,590	6,24,40,221
TOTAL (A)		**21,64,48,982**	**21,62,63,240**
EXPENDITURE			
Establishment Expenses	8	7,26,67,976	6,42,01,783
Other Administrative Expenses etc.	9	9,83,26,167	11,23,42,321
Excess of Expenditure Carried over	5(c 7)	(2,38,18,751)	(2,27,21,085)
Depreciation (Net Total at the year end)	4	6,92,73,590	6,24,40,221
TOTAL(B)		**21,64,48,982**	**21,62,63,240**
Balance being excess of Income Over Expenditure (A-B)		-	
Transfer to special Reserve(Specify each)		-	
Transfer to /from General Reserve		-	
BALANCE BEING SURPLUS (DEFICIT) CARRIED TO CORPUS/CAPITAL FUND		-	

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

BIJU MATHEW
SENIOR MANAGER (A&F)

SUDHANSHU VRATI
EXECUTIVE DIRECTOR

RASHMI GUPTA
PARTNER

PLACE: Faridabad
DATE: 27/09/2017

Regional Centre for Biotechnology

Accounting Policies and Notes Forming Parts of the Balance Sheet and Income & Expenditure Account for the Year Ended at 31st March, 2017

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 thereafter, the bill for enactment of RCB by the Parliament was enacted in July 2016. The bill was notified on 1st March 2017. 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 new and have been taken over by the new entity on the date of formation.

3. Since the RCB bill has been passed, as on the date of signing the audit report, the liabilities on account of provisions for Gratuity & terminal benefits of the Centre will worked out & incorporated in the accounts for FY 2017-18 in accordance with the approved service conditions of the RCB, which has been adopted. No provision has been made by the Institute towards the gratuity payable and other terminal benefits to staff during the FY 2016-17.

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

   (b) Grants for core funds relatable to depreciable fixed assets are treated as deferred income and recognised in the Income and Expenditure Account on a systematic and rational basis over the useful life of such assets i.e. such grants are allocated to income over the periods and in the proportions in which depreciation in charged. During the year income recognised in respect of such Grants amounts to Rs. 692,73,590.00

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

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

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

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

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

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

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

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

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

13. The Institute has received contribution of Rs. 218,65,59,416.00 (including RCB) from various institutes for the under Phase I & Phase I (Extension) of the construction of campus at Faridabad. The consolidated details are as under:

Rs. In lakhs)

Sl.No	Constituent Partner	Opening Balance as on 1.4.2016	Received during 2016-17	Total receipts on 31.3.2017
1	THSTI	9383.30	733.09	10116.39
2.	NII	1879.02	-	1879.02
3.	RCB	6500.65	-	6500.65
4.	Bio-Incubator	1320.16	750.00	2070.16
5.	ATPC	577.22	-	577.22
6.	Interest on investment of BSC funds	711.25	10.91	722.16
	Total	**20371.60**	**1493.99**	**21865.59**

and the total expenditure incurred as on 31st March 2017 against such contribution is amounted to Rs. 199,38,92,143.00 (Rs.195,19,46,843.00 being the booked as Capital Work-in-progress and & Rs.419,45,300.00 being advanced to the Project Monitoring Consultant). Although the construction is 100% completed as per the contract by EIL, the final settlement of accounts and capitalization of assets is pending submission of the final bill by EIL. The entire cost of construction is parked in CWIP as on 31.3.2017. The initial contract was

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

15. TDS Demand outstanding as on 31.03.2017 amounting Rs 1,35,040 which is not settled till the date of signing balance sheet

16. Medical Reimbursement paid to employees more than Rs.15000 is not considered for deducting TDS from Salary of the employees

17. M/s Blue Star was engaged as a contractor for CAMC services for the HVAC systems in the cluster and there is an ongoing dispute regarding the services rendered. As a result there is no provision done as on 31.03.2017

For Srivastava Kumar & Co.
Chartered Accountants

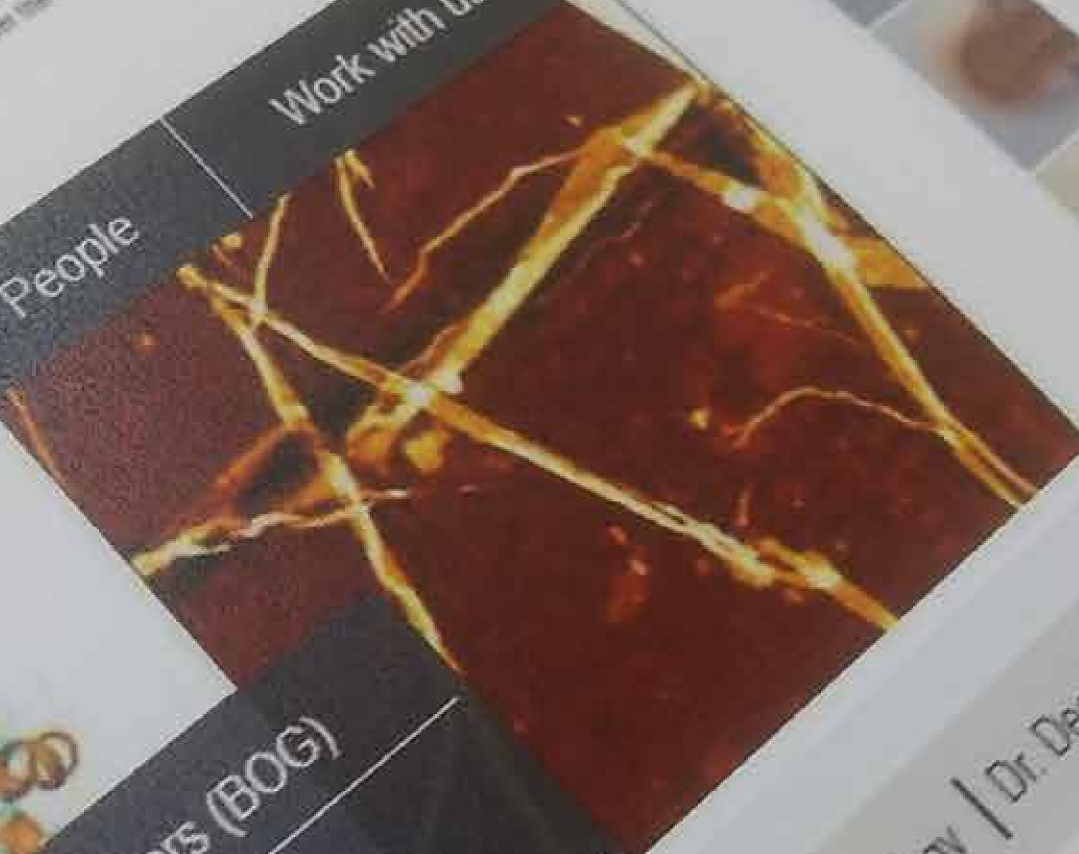
(Biju Mathew) — **Sr. Manager (A&F)**

(Dr. Sudhanshu Vratri) — **Executive Director**

Rashmi Gupta — **Partner**

Place: Faridabad
Date: 27/09/2017

Institutional Governance



Board of Governors

Prof. K. VijayRaghavan Secretary, Department of Biotechnology Block-2, 7th Floor CGO Complex, Lodhi Road New Delhi - 110 003	Chairperson
Prof. M. Radhakrishna Pillai Director, Rajiv Gandhi Centre for Biotechnology Poojappura, Thiruvananthapuram - 695 014 Kerala	Ex-officio Member
Prof. Sharmila Sengupta Director, National Institute of Biomedical Genomics Netaji Subhas Sanatorium and Tuberculosis Hospital, 2nd Floor, P.O.: N.S.S., Kalyani 741 251, West Bengal	Ex-officio Member
Prof. Gagandeep Kang Executive Director, Translational Health Science & Technology Institute NCR Biotech Science Cluster, Faridabad - 121 001	Ex-officio Member
Mr. Shigeru Aoyagi Director, UNESCO Delhi Office UNESCO House, 1, San Martin Marg, Chanakyapuri, New Delhi - 110 021	Ex-officio Member
Prof. Y. K. Gupta Professor & Head, Department of Pharmacology All India Institute of Medical Sciences, New Delhi - 110 029	Permanent Invitee
Mr. Sundeep Sarin Advisor & Nodal officer for RCB Department of Biotechnology Govt. of India New Delhi	Invitee
Prof. Sudhanshu Vrati Executive Director, Regional Centre for Biotechnology NCR Biotech Science Cluster, Faridabad - 121 001	Convenor

Programme Advisory Committee

Prof. Y. K. Gupta Professor & Head, Department of Pharmacology, AIIMS, New Delhi	Chairperson
Dr. Debashis Mitra Scientist-G & Dean (Acad) National Centre for Cell Science Ganeshkhind, Pune	Member
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Dr. Rashna Bhandari Centre for DNA Fingerprinting and Diagnostics Hyderabad	Member
Mr. Shrikumar Suryanarayan Chairman, Sea6energy Bengaluru	Member
Dr. Paramjit Khurana Department of Plant Molecular Biology, University of Delhi South Campus, New Delhi	Member
Prof. Rakesh Bhatnagar School of Biotechnology Jawaharlal Nehru University New Delhi	Member
Prof. Joel Sussman Dept. of Structural Biology The Weizmann Institute of Science, Israel	Member
Prof. Angelo Azzi Vascular Biology Laboratory Tufts University, USA	Member
Prof. R. Venkata Rao Vice Chancellor National Law School of India University Bangalore	Member
Mr. Sundeep Sarin Advisor & Nodal officer for RCB Department of Biotechnology Govt. of India, New Delhi	Invitee
Prof. Sudhanshu Vrati Executive Director RCB, Faridabad	Member Secretary

Executive Committee

Prof. Sudhanshu Vrati Executive Director Regional Centre for Biotechnology, Faridabad	Chairman, Ex-officio
Deans Regional Centre for Biotechnology, Faridabad	Members, Ex-officio
Mr. Chandra Prakash Goyal Joint Secretary (Administration) Department of Biotechnology Govt. of India, New Delhi	Member, Ex-officio
Mr. Shigeru Aoyagi Director & UNESCO representative to Bhutan India, Maldives and Sri Lanka UNESCO Office, New Delhi	Member, Ex-officio
Mr. Sundeep Sarin Advisor, Nodal officer from DBT Department of Biotechnology Govt. of India, New Delhi	Member, Ex-officio
Joint Secretary (IC) Dept. of Higher Education Ministry of Human Resource Development Govt. of India, New Delhi	Member, Ex-officio
Joint Secretary UNES Division Ministry Of External Affairs Govt. of India, New Delhi	Member, Ex-officio
Registrar Regional Centre for Biotechnology, Faridabad	Permanent Invitee
Finance Officer Regional Centre for Biotechnology, Faridabad	Permanent Invitee
Controller of Administration Regional Centre for Biotechnology, Faridabad	Member Secretary, Ex-officio

Finance Committee

Prof. Sudhanshu Vrati Executive Director Regional Centre for Biotechnology, Faridabad	Chairman, Ex-officio
Ms. Gargi Kaul Joint Secretary & Financial Advisor Department of Biotechnology	Member, Ex-officio
Mr. Sundeep Sarin Advisor, Nodal officer from DBT Department of Biotechnology Govt. of India, New Delhi	Member, Ex-officio
Dr. Gagandeep Kang Executive Director, Translational Health Science and Technology Institute Faridabad	Member, Ex-officio
Dr. Sandeep Chatterjee Registrar, IIT-Delhi	Nominated Member
Mr. Pitambar Behera Sr. Finance Officer Indian Institute of Foreign Trade, New Delhi	Nominated Member
Controller of Administration Regional Centre for Biotechnology Faridabad	Member, Ex-officio
Sr. Manager (Administration & Finance) Regional Centre for Biotechnology Faridabad	Member Secretary, Ex-officio

Scientific Personnel

Faculty

Executive Director
Prof. Sudhanshu Vrati

Professor
Dr. Prasenjit Guchhait

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

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

Honorary Visiting Scientist
Prof. S. V. Eswaran

DST INSPIRE Faculty
Dr. Megha Kumar

Wellcome Trust-DBT IA Intermediate Fellow
Dr. Pinky K. Sharma

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

International Adjunct Faculty
Prof. Falguni Sen

Young Investigators
Dr. Masum Saini
Dr. Sheetal Chawla
Dr. Amit Kumar Yadav
Dr. Vaibhav Kumar Pandya
Dr. Rashi Gupta
Dr. Sunil Kumar Tripathi
Dr. Siddhi Gupta
Dr. Shivendra Pratap
Dr. Prabhakar M.
Dr. Yashika Walia Dhir
Dr. Kuldeep Verma
Dr. Minu Nain

Wellcome Trust-DBT IA Early Career Fellow
Dr. Pushpa Kumari

Research Fellows (PhD Scholars)

Sagar Mahale
Vedagopuram Sreekanth
Harsh Kumar
Harmeet Kaur
Pranita Hanpude
Pergu Rajaiah
Gowtham Kumar Annarapu
Somnath Kundu
Gayatree Mohapatra
Roshan Kumar
Amit Sharma
Priyanka Chaurasia
Sarita Chandan Sharma
Salman Ahmad Mustfa
Rashi Singhal
Kavita Yadav
Chanchal
Hitika Gulabani
Angika Bhasym
Megha Agarwal
Tanu Johari
Amrita Kumari
Abhiruchi Kant
Nihal Medatwal
Sanjay Kumar
Pankaj Kumar
Sandeep Kumar
Amrita Ojha
Sarika Rana
Sheenam
Manhar Singh Rawat
Jithesh Kottur
Naveen Narayanan
Sanjay Pal
Sunayana Dagar
Abhin Kumar Megta
Akashi
Sulagna Bhattacharya
Meha Shikhi
Priyajit Banerjee
Syed Mohd. Aamir Suhail
Rajnesh Kumari Yadav
Ingole Kishore Dnyaneshwar
Megha Gupta
Pharvendra Kumar
Minakshi Sharma
Raniki Kumari
Rahul Sharma
Shivlee Nirwal
Shilpi Nagpal
Mary K. Johnson
Hridya Chandrasekar
Shreyasi Das
Manisha Kumari
Arunima Gupta
Krishnendu Goswami
Mritunjay Kasera
Zaid Kamal Madni
Chandan Kumar
Swatee Saberi Upadhyay
Akriti Sharma
Shrimali Nishith Maheshbhai
Sandhini Saha
Shraddha Kantilal Dahale
Animesh Kar
Anushree
Preksha Gaur
Sonalika Maurya
Priyanka Verma
Saibal Saha
Patterson Clement C
Smita Yadav
Jaya Saini
Amar Prajapati
Pankaj Kumar Sahoo
Arundhati Deb

Project Fellows

Senior Research Fellow
Abhishek Kumar Singh

Junior Research Fellows
Malyla Vamshikrishna
Deepak Kumar Mishra
Mohammad Asad
Varsha Gupta
Ankit Gupta
Divya Saxena
Parul Rani
Anil Kumar Singh
Akanksha Verma
Surbhi Mittal
Rituparna Basak
Himani Joshi

Research Associates/ Post-Doctoral Fellows
Jewel Jameeta Noor
Madhurima Mitra
Gunjan Sharma
Sameer Gupta
Amit Kumar Rajora
Amit Kumar Dey
Bhoj Kumar
Sagar P. Mahale
Tapas Bhattacharya
Priyanka Parijat

Project Assistants
Aakanksha Verma
Harsh Kumar
Neha
Teena Bhakuni

Project Associate
Shrishti Sanghi

Project Scientist
Sachin Kumar

Lab Attendent
Bhumika Arora

Bioinformatics Expert
Sachin Bhatt

Shashank Kumar Sharma

Senior Technology Officer
Sharad Vashisht

Field Operating officer
Abha Jain

Management

Executive Director
Prof. Sudhanshu Vrati

Staff Officer to Executive Director
Dr. Nidhi Sharma

Academics

Registrar

Documentation Assistant
Mr. Deepak Kumar

Management Assistant
Mr. Chakrawan Singh Chahar

Administration & Finance

Executive Director
Prof. Sudhanshu Vrati

Senior Manager (A&F)
Mr. Biju Mathew

Administrative Officer
Mr. V.M.S. Gandhi

Section Officer
Mr. Rakesh Kumar Yadav

Management Assistants
Mr. Sanjeev Kumar Rana
Mr. Sudhir Kumar

Technical Officer
Mr. Mahfooz Alam

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

Engineering

Executive Engineer
Mr. R.K. Rathore

Consultants

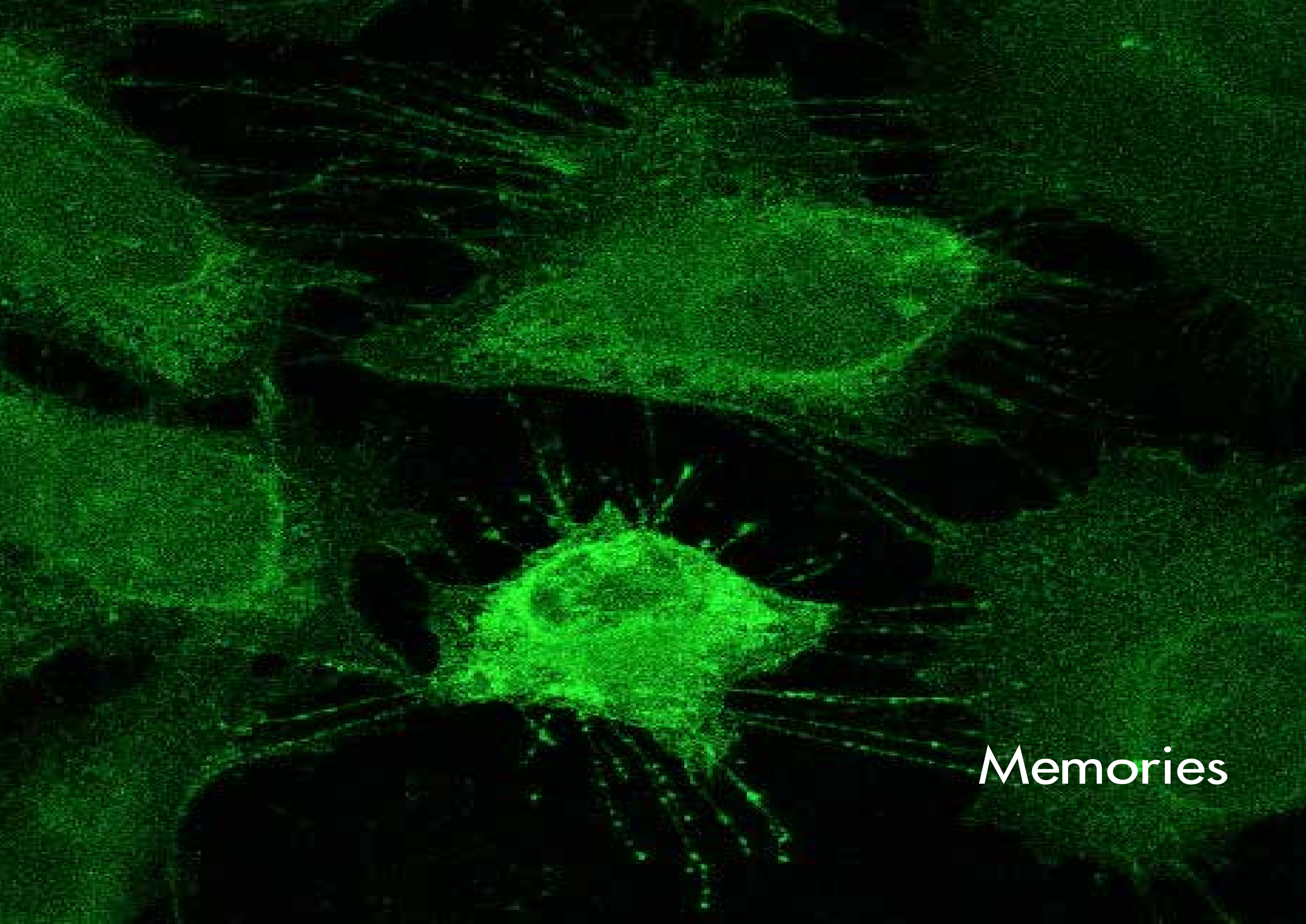
Scientific & Technical
Dr. Nirpendra Singh

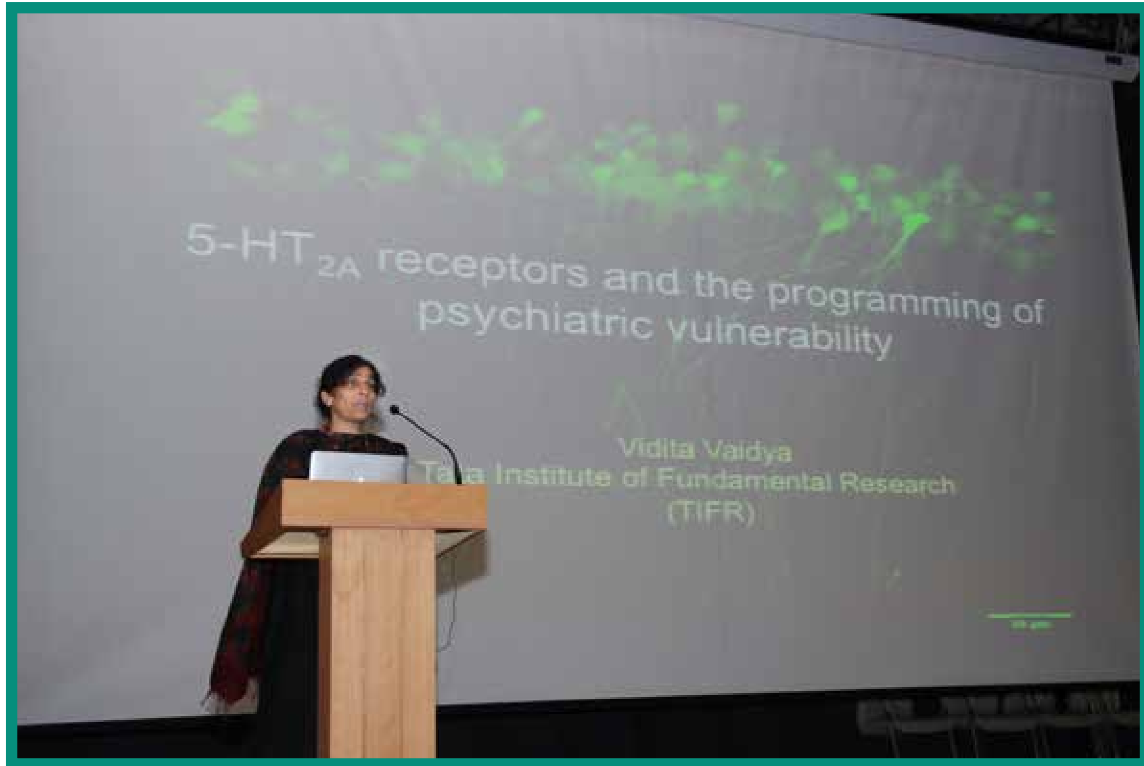
Junior Consultant
Ms. Nikita Siwach

Finance
Mr. C.L. Raina

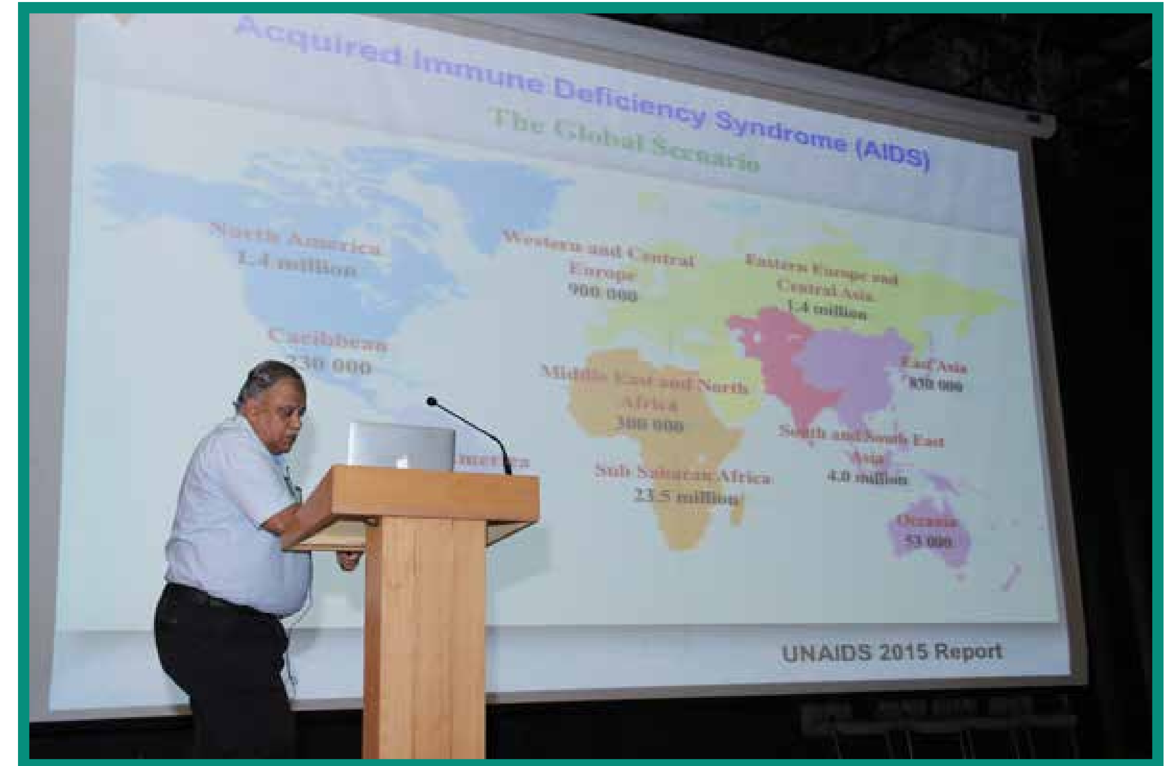
Engineering
Mr. S.S. Budhwar

Information Technology
Ms. Alka Chug


Memories



Dr. Vidita Vaidya (TIFR, Mumbai) delivering colloquium lecture



Dr. Debashis Mitra (NCCS, Pune) delivering colloquium lecture

Dr. Rashna Bhandari (CDFD, Hyderabad) delivering colloquium lecture

Poster session by RCB researchers

Prof. Seyed E. Hasnain (Jamia Hamdard, New Delhi) delivering the 'Distinguished Lecture'

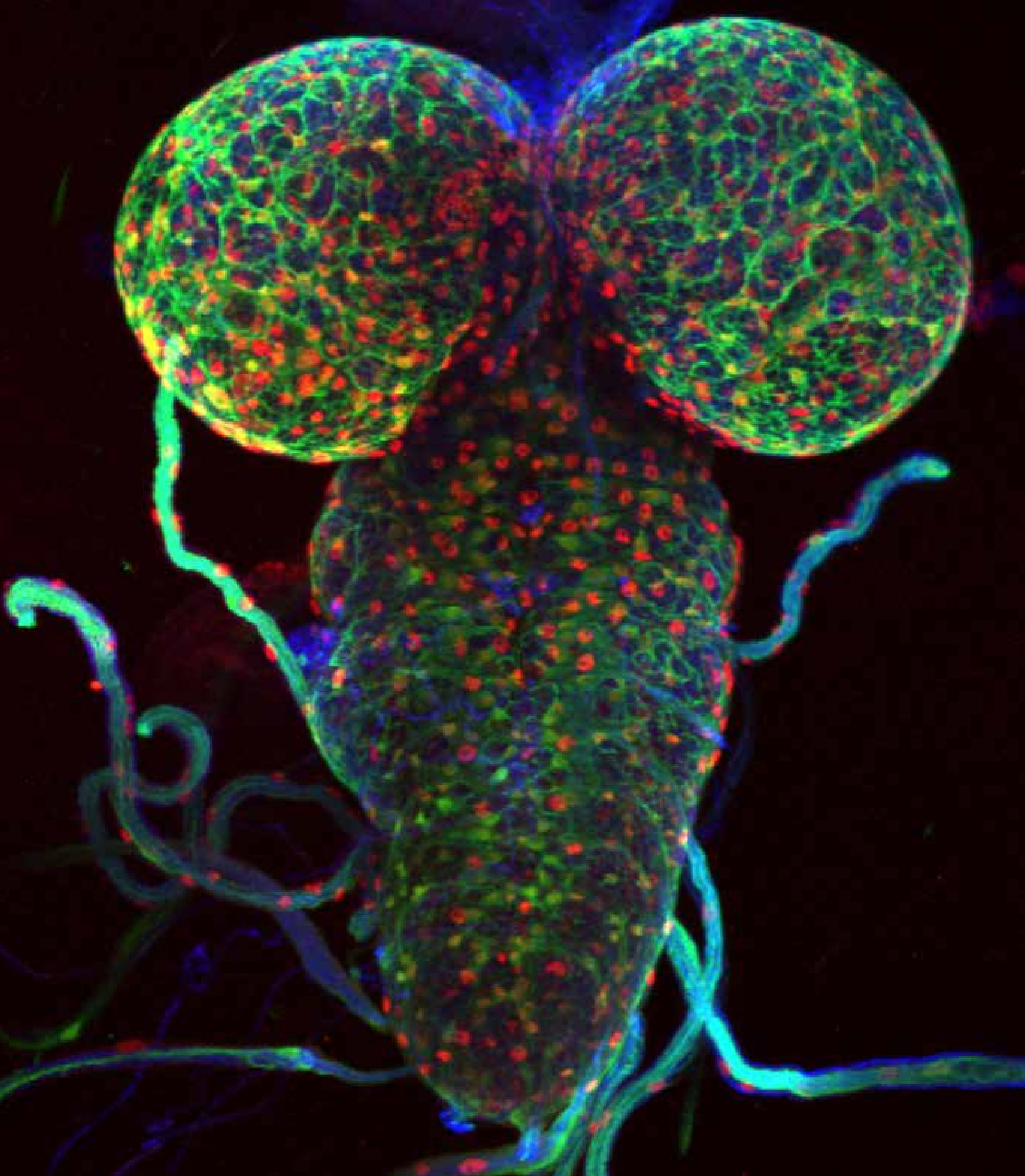
Amritra Kumari (PhD Scholar) won the gold medal at 4th AIST International Imaging Workshop held at BRI, Tsukuba, Japan

Dr. Deepak T. Nair (RCB) being honored for his 'Shanti Swarup Bhatnagar Prize', 2017 awarded by Council for Scientific and Industrial Research (Government of India)

Dr. Abha Jain, first PhD scholar to graduate from RCB

भारत का राजपत्र

The Gazette of India



REGIONAL CENTRE FOR BIOTECHNOLOGY

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