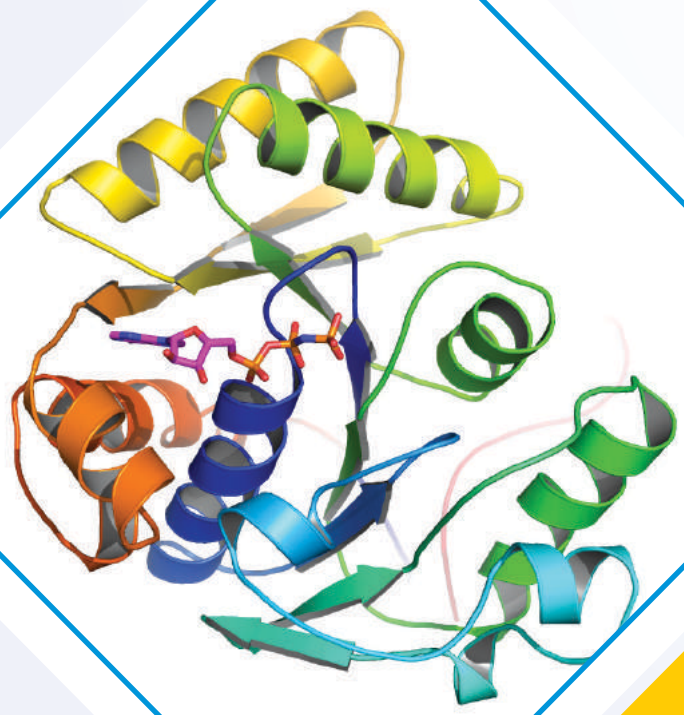
United Nations
Educational, Scientific and
Cultural Organization

क्षेत्रीय जैव प्रौद्योगिकी केन्द्र
Regional Centre
for Biotechnology



ANNUAL REPORT

2017-2018


RCB
REGIONAL CENTRE FOR BIOTECHNOLOGY

Contents

From the Executive Director's Desk

Members of the Regional Centre for Biotechnology (RCB) have continued to work tirelessly towards achieving its goals in the academic as well as the R&D spheres. Following the approval of the RCB statutes, ordinances and regulations, an integrated MSc-PhD programme in Biotechnology was launched where students with a graduate degree shall be admitted through a nationally-competitive process. We expect to admit up to 20 students to this program in the coming months. The RCB Act 2016 also empowers the Centre to recognize institutions of higher learning for their various academic programs and in this direction, after the due diligence, RCB granted academic recognition to the PhD programs at the Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad; National Institute of Animal Biotechnology (NIAB), Hyderabad; National Agri-Biotechnology Institute (NABI), Mohali; and Centre for Innovative and Applied Biotechnology (CIAB), Mohali.

RCB provides opportunities for young scientists by offering short-term innovative education and training programmes that contribute to the human resource development in the advanced areas of life sciences and biotech sciences. In this direction, the first RCB Bioimaging School was conducted during March 2018. The school highlighted popular imaging systems that have been extensively used in biology and biomedicine. Twenty-one participants from across the spectrum of research scholars, postdoctoral fellows and faculty were selected for the school from across India. The instructors and speakers included experts from prestigious academic research institutions and universities in India and Japan. The Biosafety Support Unit (BSU) of the Centre trained Biosafety regulators from different African countries on 'Food/Feed & Environmental Risk Assessment-as part of the Biotechnology and Biosafety study tour to India for African nationals in February 2018 in association with TERI. The third meeting of the India-US Strategic Dialogue on Biosecurity was organized in February 2018 where experts from both the countries reviewed Biosecurity issues, potential biological threats and our preparedness.

Under the auspices of the Department of Biotechnology (DBT) of the 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. The program was launched by the Honorable Minister for Science and Technology Dr. Harsh Vardhan in May 2017. This programme has provided tremendous support to the Indian structural biologists and has benefited a large number of young research students.

RCB continues to be a category-2 institution of the UNESCO, the linkage providing an international reach to our academic and training programmes. A review of the Asian Biotechnology School program of UNESCO was conducted at RCB in October 2017, where partners of the program from Japan, Indonesia, the Philippines, Thailand, Malaysia, Vietnam and Myanmar deliberated upon the outcome of the program and the future possibilities.

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. Several advances were made in the various research areas being pursued at the Centre. 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.

Rhabdomyosarcoma (RMS) is a predominantly pediatric soft-tissue cancer where the tumor cells have characteristics of the developing skeletal muscle. The two most common RMS sub-types are embryonal and alveolar RMS. Elevated activation of the receptor tyrosine kinase (RTK) MET is frequent in RMS and is thought to cause increased tumor metastasis and lack of differentiation. However, the reasons underlying dysregulated MET expression and activation in RMS are not well understood. Dr. Sam Mathew's group studied the role of Sprouty 2 (SPRY2), a modulator of RTK signaling, in regulating MET. Their data indicate that SPRY2 interacts with MET and stabilizes it in order to maintain signaling downstream of MET, which keeps the ERK/MAPK pathway active, resulting in metastatic potential and inhibition of differentiation in RMS. These results identify a novel mechanism by which MET signaling is stabilized in RMS, and is a potential target for therapeutic intervention in RMS.

Weakly basic drugs display poor solubility and tend to precipitate in the stomach's acidic environment causing reduced oral bioavailability. Dr. Avinash Bajaj's group designed a gastric pH stable bile acid-derived amphiphile where Tamoxifen (as a model anticancer drug) is conjugated to lithocholic acid-derived phospholipid. *In vitro* studies suggested the selective nature of conjugated drug for cancer cells and an increased intracellular uptake compared to Tamoxifen. Further studies showed a significantly enhanced anti-tumor activity and improved pharmacokinetic properties of the conjugated drug. These studies provide a new platform for oral delivery and tracing of chemotherapeutic drugs.

Additionally, 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.

Finally, I would like to place on record the excellent 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,

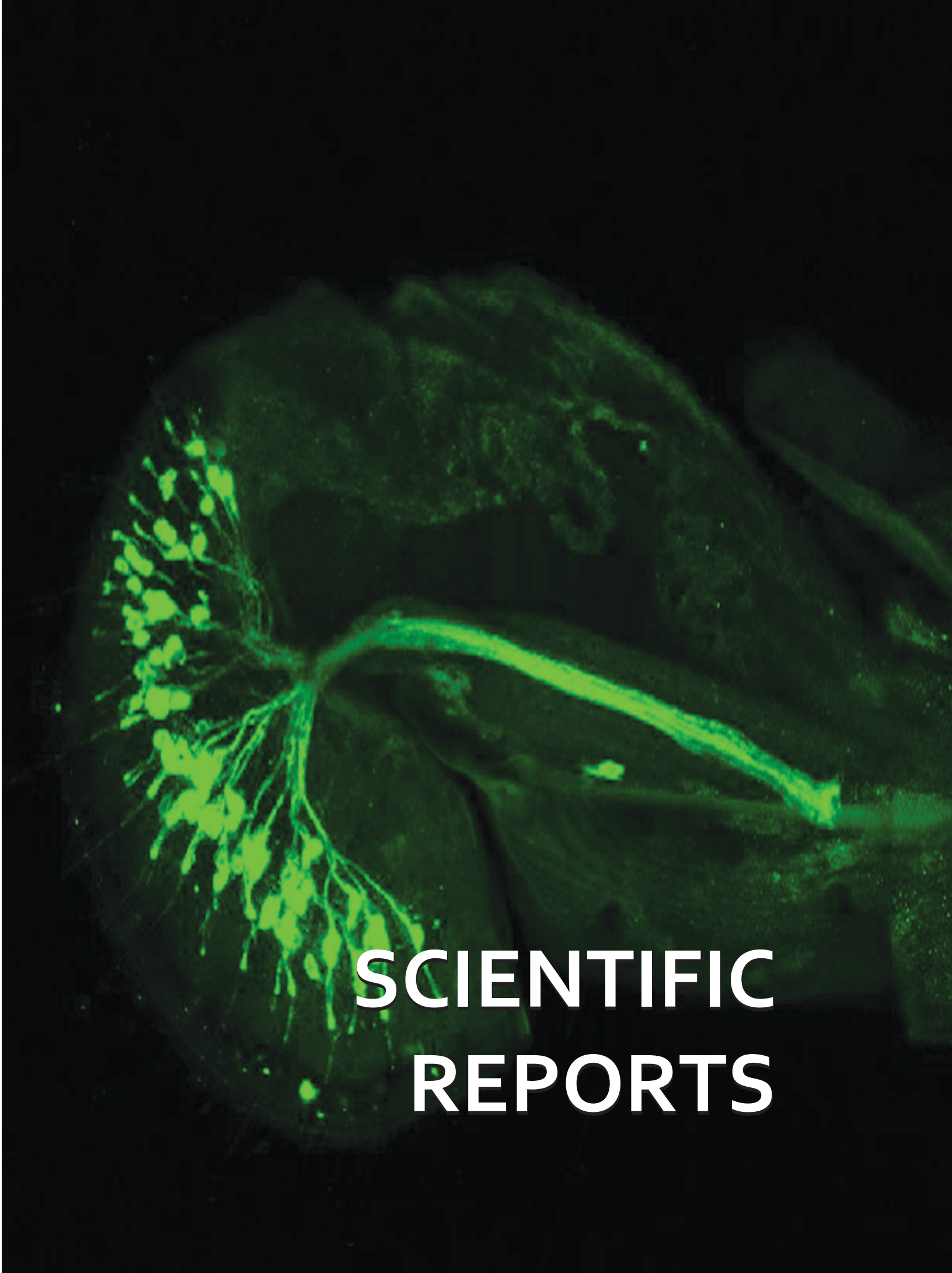
f. to collect universally available information with a view to setting up data banks for bio-information,

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

Dr. Deepak T. Nair

Principal Investigator

Co-Investigators

D. N. Rao, IISc, Bengaluru
Pradeepkumar, PI, IIT Bombay

Group members

Vaibhav Pandya
Nishant K. Varshney
Jithesh Kottur
Shivlee Nirwal
Rahul Sharma
Naveen Narayanan
Mary K. Johnson
Shilpi Nagpal
Minakshi Sharma
Patterson Clement

The genome houses the blueprint of life and any changes in this blueprint - in the form of mutations - are generally harmful. Conversely, mutations are necessary for the appearance of beneficial variations under conditions of stress to aid the survival of living organisms. We aim to understand how the integrity of the genome is maintained and also unearth the underlying causes for the appearance of variations in the genomic blueprint. The ability of pathogens to evolve under conditions of stress is responsible for the onset of multi-drug resistance and the failure of vaccines. The insight obtained from studies conducted in our laboratory will enable development of novel therapeutic strategies to combat antimicrobial resistance and virus infections, two major public health problems that plague the world currently.

We study molecules involved in the maintenance of genetic integrity or the appearance of genomic plasticity. Our efforts ultimately provide insight into the processes that enable pathogenic organisms to adapt and gain resistance to therapeutic and prophylactic agents.

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 antagonistic action of these two different sets of molecules ensures that genomic plasticity is calibrated to endow adaptive capability without severely compromising genetic viability. Since therapeutic agents exert strong selection pressure on pathogens, genomic plasticity is implicated in the onset of drug resistance and reduction in vaccine efficacy. We aim to elucidate the structural mechanism utilized by different molecular determinants of genomic integrity and plasticity to achieve function. With this broad aim in mind, the biological processes under scrutiny in our laboratory are (a) DNA replication, (b) Stress-induced mutagenesis, (c) DNA mismatch repair, (d) Stress-induced epigenetic modification, (e) Transposition, and (f) Replication of the genome of Japanese Encephalitis (JEV) and Chikungunya viruses. Our efforts will provide insight into how the genome is accurately duplicated and how variation arises in the genotype and phenotype of organisms, especially in response to an adverse environment. The insight gained from these studies will provide a robust platform for

the development of novel therapeutic strategies that target molecular processes that enable genome duplication or adaptation in pathogenic bacteria and viruses. The progress made in some of these projects in the past year is presented below.

DNA Replication

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. $Mg2+$ ions also play an important role in the polymerization reaction. DNA polymerases extend the primer in the 5'-3' direction. The formation of a phosphodiester bond between the α-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.

We have conducted time-resolved crystallography 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. This study shows that hydrolysis of the PPi byproduct is an intrinsic and critical step of the DNA synthesis reaction. Biochemical assays with appropriately modified dNTPs suggest that this step may be conserved in different DNA polymerases, including the RNA-dependent-DNA polymerase responsible for duplication of the RNA genome of retroviruses such as HIV.

These studies dispel a long-standing belief that DNA synthesis is an example of a coupled reaction wherein the byproduct PPi moiety is hydrolysed by accompanying pyrophosphatase enzymes to render a large negative free energy to the overall reaction. Our study shows that the hydrolysis of PPi occurs after the formation of the phosphodiester bond and ensures that the DNA synthesis reaction is

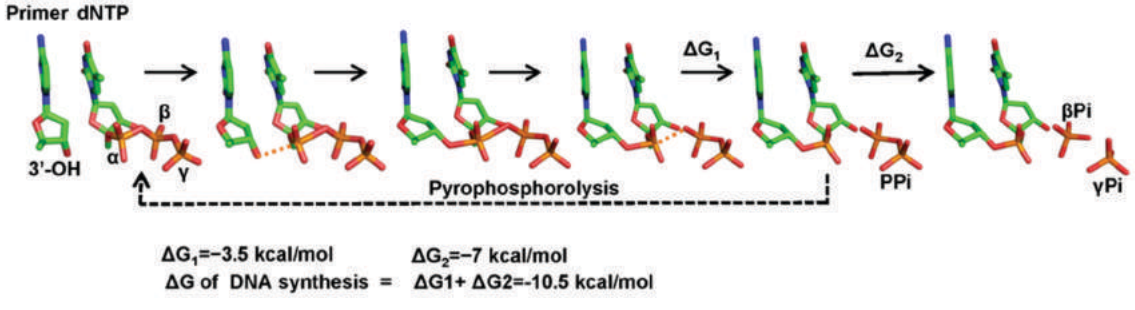


Figure 1: Mechanism of DNA synthesis: *The hydrolysis of PPi by dPols to phosphate ions is an intrinsic and critical part of the DNA synthesis reaction. This step ensures that the reaction is energetically favourable and moves in the forward direction without the need for additional enzymes.*

energetically favorable without the need for additional enzymes (Fig. 1). Overall, the study brings to light the mechanism of the fundamental reaction responsible for genome duplication and the insight obtained from this study may aid the development of improved PCR-based diagnostic kits and novel therapeutic strategies against retroviruses.

DNA Mismatch Repair

The Mismatch Repair (MMR) Pathway serves to maintain genomic integrity by correcting errors that appear during replication. Although MMR in *E. coli* is reasonably well-characterized, it is known that the majority of bacteria and all eukaryotes do not follow a similar pathway. Using MMR in *Neisseria gonorrhoeae* (Ngo) as a model system, we aim to elucidate the mechanism of MMR in organisms that

do not follow the *E. coli* paradigm. In Ngo, the specific proteins associated with MMR are represented by NgoS (ortholog of MutS) and NgoL (ortholog of MutL).

MutS represents the primary mismatch sensor and forms a dimer clamp that encircles DNA and bends it to scan for mismatches. Two monomers of MutS associate to form an oval disc-shaped asymmetric dimer with a central channel into which DNA is loaded. MutS shows the presence of four regions, the N-terminal domain (NTD), the central domain, clamp region and the C-terminal domain (CTD). The mechanism by which the MutS dimer encircles DNA was not known, and the origin of force required to bend DNA was unclear.

We show that in the absence of DNA and presence of ADP or AMPPNP, NgoS forms a symmetric dimer wherein the two monomers are associated only through the CTD, and there is no interaction between the clamp regions. Consequently, a large gap exists between the clamp regions through which DNA can enter the central channel. The mismatch scanning monomer (Bm) then moves by nearly 50 Å to associate with the other monomer (Am) so that the clamp regions come in contact with each other and the dimer encircles DNA. Due to the movement of Bm, 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 Bm and hence, the MutS dimer acts like a pair of pliers to bend DNA (Fig. 2).

Also, our study shows that the ATP binding and not hydrolysis is critical for formation of the MutS-DNA complex and that the ATP molecule bound to Am is expelled on DNA binding. Overall, this study

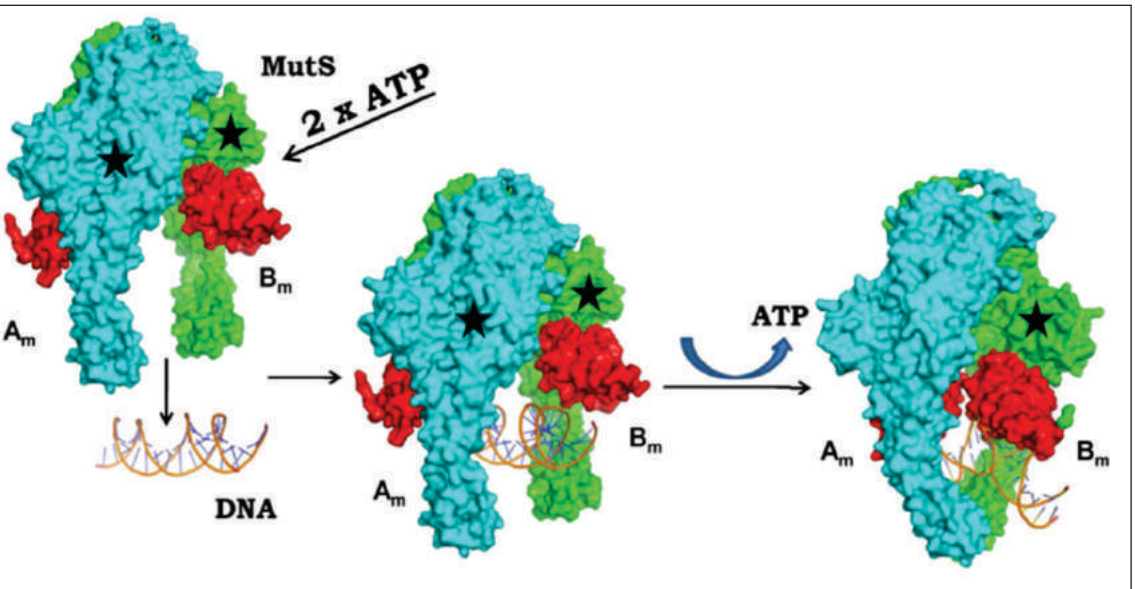


Figure 2: Mechanism of assembly of the MutS-DNA complex: *The different stages in the assembly of MutS-DNA complex are displayed. The two monomers are shown in surface representation, labeled Am and Bm and are colored cyan and green, respectively. The N-terminal domain of each monomer is coloured red and black star represents ATP molecules. Our study shows that the MutS dimer acts like a pair of pliers to bend DNA.*

provides mechanistic insight regarding the primary event in DNA mismatch repair i.e. the assembly of the MutS-DNA complex. The insight gained from this study can be exploited to develop small molecule inhibitors of the MMR pathway. It is predicted that inhibition of MMR will ultimately increase the frequency at which deleterious mutations appear in the genome and attenuate the ability of bacterial pathogens to proliferate and cause disease.

Transposition

Transposons are mobile genetic elements that give rise to variation in the genome and can be responsible for horizontal gene transfer. The movement of transposons has been implicated in the onset of drug resistance since many transposons carry genes that endow resistance to antibiotics. The mobility of these genetic elements is primarily mediated by cognate enzymes known as transposases. These enzymes mediate the excision of the transposon from the origin site, followed by translocation and integration at the target site. We aim to understand the mechanism employed by different transposases to achieve function.

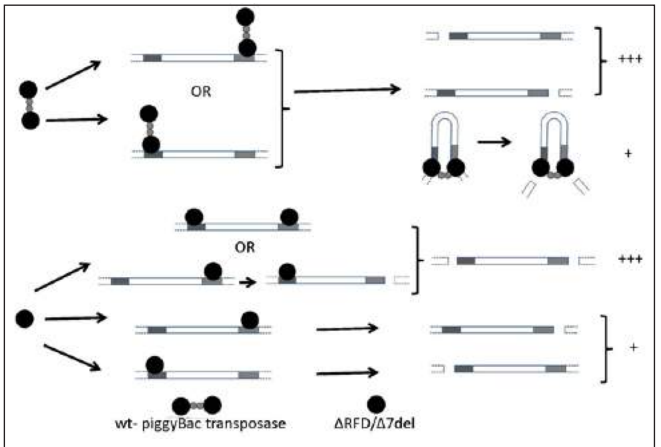


Figure 3: Dimerization attenuates the excision activity of the piggyBac transposase: *According to the studies presented here, dimerization through the last seven residues of the RFD results in a protein that exhibits reduced ability to excise the transposon. Deletion of the RFD or the terminal seven residues results in an enhancement in the ability to excise the transposon.*

The piggyBac transposon was isolated from the cabbage looper moth, and the movement of this transposon is mediated by its cognate transposase. We have shown that the RING-finger domain (RFD) present toward the C-terminus of the transposase is vital for dimerization of this enzyme. The deletion of the RFD or the last seven residues of the RFD results in a monomeric protein that binds the terminal end of the transposon with nearly the same affinity as wild-type piggyBac transposase. Surprisingly, the monomeric constructs exhibit greater than 2-fold enhancement in the excision activity of the enzyme. Overall, our studies suggest that dimerization attenuates the excision activity of the piggyBac transposase (Fig. 3) and may serve to prevent excessive transposition of the piggyBac transposon that might be catastrophic for the host cell. The piggyBac transposon is utilized as a genome engineering tool and is employed in the transformation of differentiated cells into pleuripotent stem cells. Our studies may help in the development of hyperactive variants of piggyBac that can achieve this transformation with higher efficiency.

Biology of Infectious and Idiopathic Inflammation of the Gut

Dr. Chittur V. Srikanth

Principal Investigator

Group members

Prabhakar M.
Gayatree Mohapatra
Salman Mustfa Ahmad
Sarika Rana
Aamir Suhail
Pharvendra Kumar
Hridya Chandrasekar
Preksha Gaur
Sonalika Maurya
Gayatree Mohapatra

Modern ways of living have led to a sudden rise in abnormal immune function leading to auto-immune disorders. This may happen due to infection by food borne pathogens such as *Salmonella typhimurium*. Inflammatory bowel disease (IBD) is an autoimmune disorder of gastrointestinal (GI) tract involving chronic abnormal immune activation. Symptoms include recurrent diarrhea, abdominal cramps, weight loss, fatigue and a terribly compromised 'quality of life'. Several molecular pathways have been tested for therapeutic interventions but none have been found fully successful. In our study we have investigated possible role for SUMOylation, an inherent post-translational modification pathway of proteins, in IBD. In mice model and human patients, we demonstrate existence of an aberrant SUMOylation pathway linked to aberrant immune activation. In episodes of gut inflammation resulting from IBD or *Salmonella* infection we saw a common link of SUMOylation alteration. These findings, the first of their kind in gut inflammation, connect immune cell conditioning to SUMOylation and highlight its importance for therapeutic interventions.

This research programme is focused on understanding the molecular mechanisms that govern infection, inflammation and autoimmune disorders of the gut. Using a variety of systems including the cell culture model, the mouse model and human patient samples we intend to understand novel molecular mechanisms, particularly those linked to post-translational modification pathways (PTM), that possibly govern inflammation in various forms of gut illnesses. The ultimate aim is to identify novel molecular targets for possible therapeutic interventions.

As a part of this program we are pursuing studies on inflammatory diseases of the gut that arise due to (a) *Salmonella* infection, and (b) immune dysfunction such as Crohn's disease and Ulcerative colitis. Specifically, we investigate the significance and exact role of SUMOylation in Inflammatory Bowel Disease (IBD) and *Salmonella* disease. We also explore possibilities of using components of the SUMOylation machinery, or other pathways that are linked to SUMOylation, as potential targets for therapeutic interventions against gut inflammation.

Host SUMOylation governs *Salmonella* infection

Among the various microbial threats that pose a challenge to the host health, a frequent causal agent of gastric illnesses is *Salmonella typhimurium* (hereafter referred to as *Salmonella*). The disease is called gastroenteritis, accompanied with symptoms like diarrhoea, acute inflammation, fever and abdominal cramps. The recent emergence of multidrug resistant strains of *Salmonellae* has resulted in severe outcomes, thus posing a significant health challenge in the developing and developed world. Remarkably, some of the symptoms of *Salmonella*-induced gastroenteritis

are also seen in autoimmune disorders of the gut such as Crohn's disease (CD) and Ulcerative colitis (UC).

Salmonella is able to invade host cells as a result of two sophisticated type three secretion systems (TTSS) encoded by its pathogenicity islands SPI1 and SPI2. After entering the host cells the bacterium resides within a membranous pouch called *Salmonella* containing vacuoles (SCVs), which are absolutely critical for intracellular bacterial survival. How the pathogen is able to hijack the entire machineries of the host has been a long standing unanswered question.

Over the years we have shown that *Salmonella* utilises a post-translational modification (PTM) pathway of the host, called SUMOylation for successful infection (Verma *et al., Mol Cell Biol*, 2015). *Salmonella* targets multiple components of the SUMO machinery, including the E2 enzyme Ubc9 and E3 enzyme PIAS1 to take control over the pathway. Using multiple approaches, we were able to pinpoint that the decrease of Ubc9 was due to the presence of a miRNA (miR30c and miR30e). The biological significance of this phenomenon appeared be mainly to gain better bacterial intracellular multiplication. The outcome of experimental perturbation of host cell SUMOylation pathway was probed in greater detail and some of the interesting findings are presented here.

(a) The SUMOylation machinery modulates intracellular multiplication of Salmonella

In our previous study, we had tested the effect of SUMOylation only during early stages of infection. Here we tested for the possible involvement of the SUMOylation machinery at later stages of infection. Epithelial cells (HCT8) were transfected with plasmids encoding individual components of the SUMOylation pathway (such as Ubc9, SUMO1 SUMO2). These cells displayed an overall activation of the SUMOylation machinery as seen by an upregulation of the global SUMOylation profile of the cell (Fig. 4A). We checked the effect of over-activating SUMOylation on the multiplication of *Salmonella.* The number of intracellular *Salmonella* was scored by using colony forming units (CFU) assays. SUMOylation perturbation at 7 hrs and 24 hrs post-infection (pi), led to significantly compromised growth of bacteria. Specifically, at 7 hrs pi (Fig. 4B), in SUMO-perturbed cells, about 30% lowering in bacterial number was seen compared to vector control cells (VC). This difference was even more dramatic at 24 hrs pi wherein CFU in SUMO-perturbed cells descended to 50% of that from VC cells. To further understand the phenomenon, we probed the *Salmonella* induced filaments (SIFs) formation in control and SUMO-perturbed cells using confocal microscopy. SIFs are decorated by lysosomal glycoproteins (such as LAMP1) and are needed for the intracellular multiplication of *Salmonella*. In healthy cells infected with *Salmonella* we saw multiple SIFs. However, SUMO-perturbation (in this case SUMO2/3) led to a dramatic reduction in SIF formation (Fig. 4C). Since SUMOylation appeared to be governing the intracellular fate of *Salmonella*, we next set forth to decipher the SUMO-modified proteome (SUMOylome) of a *Salmonella* infected cell. We

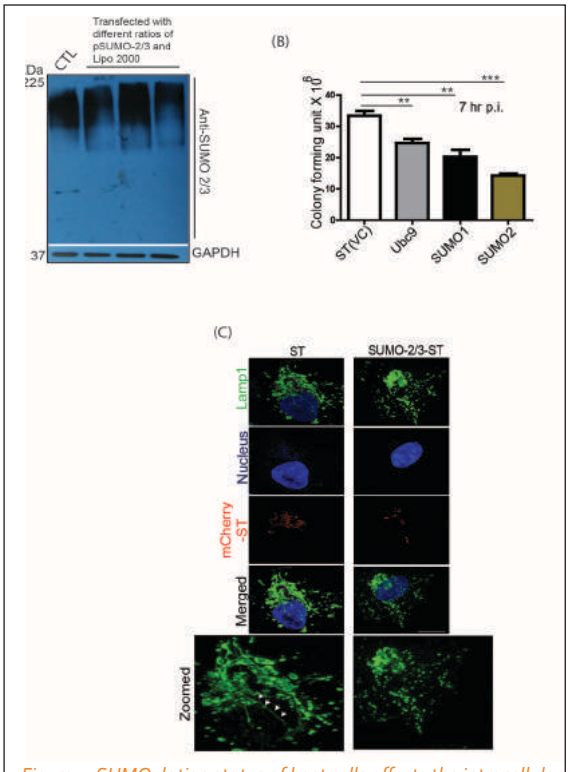


Figure 4: SUMOylation status of host cells affects the intracellular growth of Salmonella.

used a SUMO-specific affinity purification system to isolate the SUMOylome.

The proteins present in the isolated SUMOylome were identified by tandem mass-spectrometry (MS/MS). The analysis revealed several interesting candidates, Rab7, a regulator of vesicular transport system (VTS) being one of them. Rab7 is known to play an important role in several cellular processes that depend on VTS including protein sorting, autophagy and *Salmonella* biology. We first validated Rab7 SUMOylation by using a combination of *in silico* and biochemical tests. We also carried out *in vitro* SUMOylation assays using purified Rab7 protein and were able to see SUMOylated forms of Rab7. The relevance of Rab7 SUMOylation in *Salmonella*-host crosstalk was unknown therefore we probed this aspect in greater detail.

(b) SUMOylation dependent control of Rab7 function

The possible effects of experimental SUMOylation perturbation on Rab7 and VTP proteins were tested. In both infected and control cells, perturbation of SUMOylation machinery components resulted in significant lowering of Rab7 levels, while the other members of VTS remained unaffected (Fig. 5A). This was further confirmed by densitometric analysis of the blots. Using fluorescence-activated cell sorting (FACS) methodology, we purified SCVs based on *Salmonella* that expressed a mCherry fluorescence. Lysates from these purified SCVs, when immunoblotted showed a dramatic lowering of Rab7 in SUMOylation perturbed (using YFP-SUMO-1 construct) cells compared to control untreated cells. These data led us to conclude that SUMOylation perturbation resulted in lowering of Rab7 expression and localization.

Since Rab7 protein levels were regulated by the cellular SUMOylation status, we reasoned that the SUMOylation machinery may be acting either on Rab7 synthesis, or stability. To test these possibilities, we blocked the *de novo* synthesis of Rab7 using the protein synthesis inhibitor cycloheximide. We monitored the kinetics of its existing copies in SUMOylation perturbed and infected cells. It was evident that in the absence of any fresh synthesis, the levels of Rab7 diminished with time. Interestingly in SUMOylation perturbed conditions the levels of Rab7 diminished faster than control cells. Based on these data, the rate of Rab7 degradation was plotted in the form of 'decay curves'. From the decay curves, we calculated the approximate time taken for Rab7 to reach 50% of the original levels (half life).

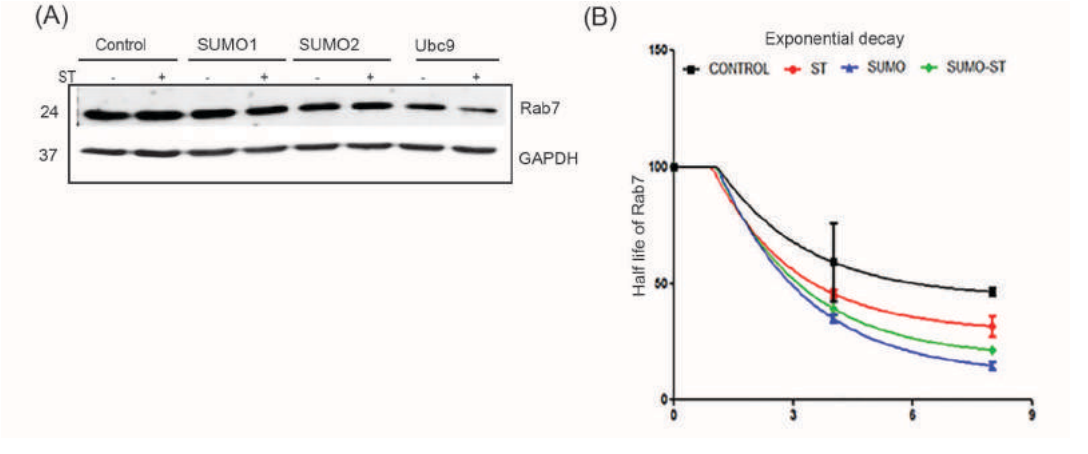


Figure 5: SUMOylation dependent modulation of Rab 7 stability.

We were able to discern that in SUMO-perturbed cells, the half-life of Rab7 was shorter (5.6 hrs) than in untreated cells (7.5 hrs, Fig. 5B). We inhibited the ubiquitin-dependent proteasome degradation machinery using the drug MG132 (a proteasome inhibitor) and examined the effect on Rab7 protein

levels. We observed that MG132 treatment was able to avert Rab7 degradation in SUMO-perturbed conditions. Together these data indicated that SUMOylation mediated Rab7-turnover occurred via the proteasome degradation machinery.

© SUMOylation of Rab7 negatively controls Salmonella multiplication

We next set forth to delve deeper into the significance of Rab7 SUMOylation altered stability on *Salmonella* biology. The various lysines of Rab7, that could potentially undergo SUMO-modification, were mutated to arginine and finally a mutant that was unable to undergo SUMOylation was generated. The SUMO-deficient mutant was named as Rab7^{SD}. To investigate the role of Rab7 SUMOylation, either of WT-Rab7 (Rab7) or SUMO-mutant Rab7^{SD} was overexpressed in HeLa cells and confocal imaging was performed (Fig. 6). During infection Rab7 is known to physically interact with *Salmonella*. In our experiments *Salmonella* was imaged using DAPI staining and Rab7 using the GFP-tag. Surprisingly, the frequency of *Salmonella* -Rab7 co-localization was significantly higher in case of the Rab7^{SD} compared to the wild type Rab7. To see if the Rab7^{SD} also displayed other features relevant to *Salmonella* infection, we tested its interaction with PLEKHM1 by immunoprecipitation. The lysates from *Salmonella* infected cells expressing Rab7 or Rab7^{SD} were immunoprecipitated and probed with PLEKHM1 antibodies. The Rab7-PLEKHM1 interaction was more in case of Rab7^{SD} compared to wildtype Rab7 indicating the importance of SUMOylation. Finally, the activity and subcellular localization of Rab7 and its SUMO deficient mutant form were examined. We observed that Rab7^{SD} was inactive and non-membranous particularly during *Salmonella* infection compared to its wild type counterpart (Fig. 7). Together these results indicate that a SUMOylation dependent mechanism controls the stability, localization and function of Rab7.

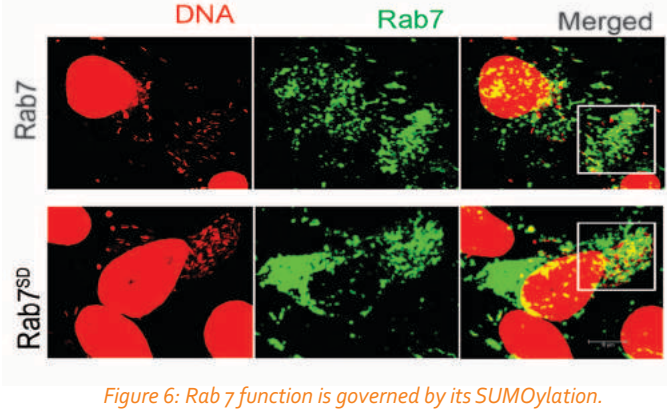


Figure 6: Rab 7 function is governed by its SUMOylation.

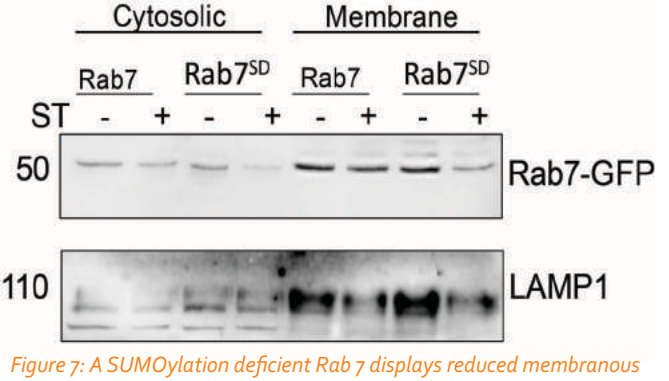


Figure 7: A SUMOylation deficient Rab 7 displays reduced membranous localization.

SUMOylation governs inflammation in inflammatory Bowel Disease (IBD)

IBD comprises of chronic relapsing inflammatory disorders and includes both Crohn's disease (CD) and Ulcerative colitis (UC). The majority of the research in IBD has gone into cellular and molecular pathways particularly in understanding immunology and genetic factors. SUMOylation has not been studied in the context of IBD at all. Our work revealed a novel connection involving epithelial SUMOylation alteration and intestinal inflammation in IBD-pathophysiology. We demonstrated that akin to *Salmonella*-induced inflammation, in IBD there was an overall lowering of the SUMOylation

pathway. The SUMOylation alteration appeared to be a critical determinant for activation of a pro-inflammatory environment. The validity of all these findings was tested by using human IBD patient biopsy samples. Together these investigations, the first of their kind, connect two very important forms of gut inflammation to SUMOylation and thus unravel novel targets for possible therapeutic interventions.

In both *Salmonella* induced inflammation and IBD we have seen an important role for cellular SUMOylation machinery. It appears that downregulation of SUMOylation macihenry is a common feature in both these forms of gut dieases. Our future goal will be to understand the precise molecular events that are operational during this process. We will be looking at key regulators to see if they are controlled by SUMO-modification. The ultimate goal is to find the key set of events that are governed by a SUMO-switch during inflammation.

Transcription Regulation: Structure and Mechanism

Dr. Deepti Jain

Principal Investigator

Group members

Amit Yadav
Neha Singh
Chanchal
Priyajit Banerjee
Pankaj Kumar Sahoo
Hemant Goswami
Shubham Dubey

Antibiotic resistance represents a global challenge in the treatment of bacterial infections. *Pseudomonas aeruginosa*, a human pathogen, notorious for hospital-borne infections exists in unicellular flagellated form and as multicellular colonies of bacteria called biofilms. Bacteria present in biofilms are resistant to antimicrobials. Our group aims to understand how the flagella and biofilm formation are regulated in the pathogenic bacteria like *Pseudomonas aeruginosa*. Understanding the regulatory pathway is essential, as it will provide a robust platform to develop novel therapeutic strategies against nosocomial pathogens such as *Psedomonas aeruginosa*.

Resistance to antibiotics represents an escalating challenge in the treatment of bacterial infections. Pathogenic bacteria are known to switch phenotype to reduce sensitivity towards antimicrobial agents. These phenotypic transitions are regulated at the level of transcription, which is an essential process responsible for gene expression. We employ an integrated approach, involving structural tools, biophysical techniques, biochemical methods and functional in vivo assays to investigate the molecular mechanisms of transcription regulation.

The mechanistic insights obtained will be exploited for the development of novel therapeutic agents against pathogenic bacteria and the development of novel inducible recombinant expression systems. The major objectives of our research programme are: (1) To study flagellar gene regulation in *Pseudomonas aeruginosa, (2)* To comprehend the mechanism of antibiotic resistance in *Staphylococcus aureus,* and (2) To elucidate the mechanisms utilized by transcription factors to achieve sensitivity towards small metabolites.

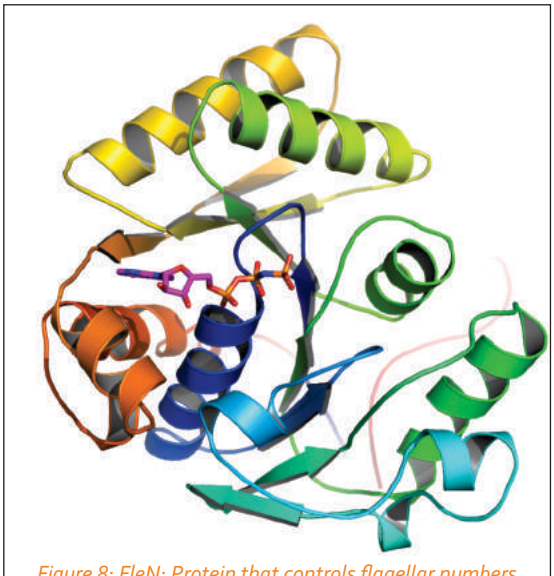


Figure 8: FleN: Protein that controls flagellar numbers

Flagellar gene network in *Pseudomonas aeruginosa*

Pseudomonas aeruoginosa (Psa) is an opportunistic human pathogen and is a major cause for hospital acquired infections. The treatment of infections caused by Psa throws a challenge due to antibiotic resistance. Therefore, there is an urgent need to discover and develop new therapeutic compounds with unique modes of action. *Pseudomonas* is known for transition from a motile to a sessile phenotype to evade antimicrobial agents. Bacterial motility is essential for colonization and invasion. Thus, proteins that regulate motility or the switch between the motile and sessile form of bacteria are attractive drug targets. These proteins form dynamic interactions with genomic DNA and other protein partners. It is essential to obtain detailed information regarding three dimensional structures of these interactions. Towards this end, we have determined the three dimensional structures of the target proteins that regulate motility in *Pseudomonas aeruginosa* and have explained in detail the mechanism of bacterial motility (Fig. 8). Currently, efforts are ongoing for development of in silico drugs that will target these regulators.

Structure-function studies of antibiotic resistance in *Staphylococcus aureus* (Sta)

Antibiotic resistance in *S. aureus* is one of the leading causes of mortality and healthcare expenditure. Thus, understanding the regulatory networks that mediate such resistance is very important. The VraSR (vancomycin resistance associated Sensor Regulator) system enhances resistance towards glycopeptide antibiotics such as vancomycin that target cell wall synthesis in *Staphyloccocus*. Surprisingly, inactivation of kinase protein of the VraSR system resulted in increased tolerance to vancomycin, raising the possibility that the response regulator was being activated by an alternative kinase, which is part of the GraSR (glycopeptide resistance associated Sensor Regulator) two component system. We aim to carry out a rigorous structural investigation of the protein-protein interactions responsible for development of resistance. We have determined the structure of the protein that plays an important role in the cross-talk between the two two-component systems. This work will aid in deciphering the putative network responsible for the increase in glycopeptide tolerance in *Staphyloccocus*. The insights obtained from this study will be used to design and test small molecule inhibitors using in silico approaches.

Allosteric mechanism utilized by transcription factors responsive towards small metabolites

Allostery has been defined as the process wherein the binding of a ligand or the effector molecule at a particular site alters the activity of the protein at a remote site. In case of transcription modulators, effector binding can either increase the affinity (activation) or can decrease its affinity to the DNA (de-repression), thereby altering the gene expression. Thus, transcription modulators serve as molecular switches, turning on and off the expression of genes. The AraR protein is the key regulatory protein of the L-arabinose metabolism in *Bacillus subtilis*. AraR is composed of two independent domains exhibiting different functions and belonging to different families of proteins. The smaller N-terminus domain (NTD), which retains its ability to bind DNA, comprises a winged helix-turn-helix motif and the larger C-terminus domain (CTD) binds L-arabinose and belongs to the LacI/GalR family. In the absence of L-arabinose, AraR binds to operator sequences and suppresses the expression of metabolic genes. Presumably, AraR undergoes a conformational change on binding L-arabinose, which releases it from cognate operators resulting in transcription initiation.

We aim to elucidate the structural basis of the allosteric mechanism utilized by AraR to abolish specific DNA recognition on arabinose binding. AraR binds to eight different operator sequences governing five different promoters and has two different modes of transcriptional repression. We have crystallized the AraR-DNA binding domain (DBD) in complex with four different natural operators and

have obtained crystals of AraR with two more. These structures shed light on the plasticity of transcription factors which endows them with the ability to tolerate differences in operator DNA sequences. This observation is in line with studies probing specificity of transcription factors using CHIP-SEQ and also sheds light on the evolution of transcription factors. We have recently obtained crystals of AraR-DBD with other operators as well and have collected data at the European Synchrotron Radiation Facility (ESRF), and are currently performing crystallographic refinement to obtain the molecular structure (Fig. 9).

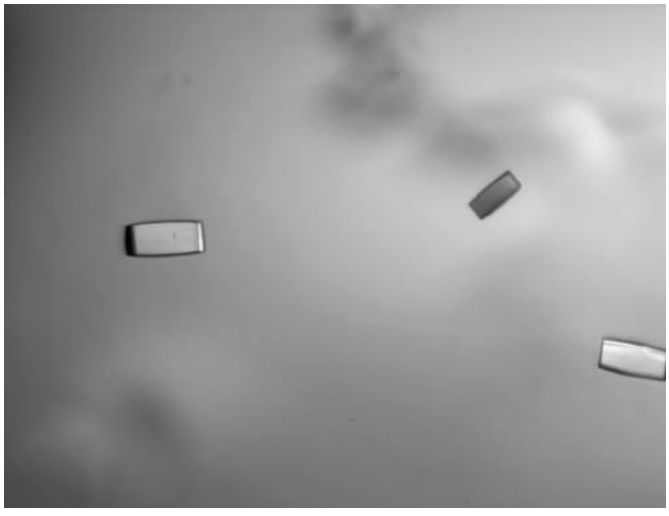


Figure 9: Crystals of AraR(NTD)-DNA complex

We have recently initiated an effort to develop an inducible expression system using engineered AraR from *Bacillus* species.

Structural Biology of Host-Microbial Interactions in Health and Diseases

Dr. Vengadesan Krishnan

Principal Investigator

Group members

Shivendra Singh
Abhiruchi Kant
Abhin Kumar Megta
Rajnesh Kumari Yadav
Smita Yadav
Amar Prajapati
Priyanka Chaurasia

Microbial attachment to the host surface is the first and key step in colonization that may benefit or damage the host based on the nature of the host-microbe relationship. Microbes often use their cell surface molecules to mediate attachment with their host. Developing drugs that can interfere with initial attachment and using good microbes to fight off the bad ones are seen as promising approaches in improving health and controlling infections. Towards providing an essential foundation for these approaches, the structural investigation programme aims to generate knowledge of mechanism by which the microbes assemble their surface adhesive molecules and interact with the host.

Our 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. The later events in the pathogenesis or probiosis are highly dependent on the success of this primary interaction. 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, we aim to generate structural knowledge of these interfaces by studying key molecules that establish the contacts between the host and microbes, both beneficial and pathogenic.

The host-microbial interface is constituted through myriad complex interactions, which primarily depend upon the success of microbial adhesion. The 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 simple task for microbes as they have to pass through multiple challenges including the host's physical and immunological clearances. To avoid being removed at the host surface in a dynamic environment, 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 has been initiated on pilus constituents from beneficial and pathogenic strains for understanding the structural basis of pilus biogenesis, architecture and pili-mediated interactions. The study was begun with some representative beneficial

strains from gut microbiota (*e.g. Lactobacillus rhamnosus* GG and *Lactobacillus ruminis*) as their knowledge is relatively limited. A few pathogenic members (*e.g.* primary colonizers of oral biofilm) were later included in the programme for getting insights into tissue tropism and understanding microbial interaction strategies in health and diseases.

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. The *L. rhamnosus* GG pili play a key role in persistence and immunomodulation for providing beneficial health effects. The *L. rhamnosus* GG utilizes pili to mediate interaction with intestinal mucus and components of extra cellular matrix (ECM). Towards understanding the molecular mechanism by which this bacterium assembles pili 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 through their respective basal minor pilins (SpaB and SpaE).

Crystal structures of SpaA that forms the SpaCBA pilus shaft, determined at a high resolution, revealed new insights about pilus shaft formation for the first time from a probiotic strain (Chaurasia *et al., Sci Rep,* 2016.). The structural analysis has added new knowledge to the field. Recent mutational analysis indicates that substitution of alanine to glutamate in the C-terminal domain initially destabilizes the fold but eventually forms the isopeptide bond with the intact fold. However, substitution with bulky and longer hydroph obic residues or positively charged residues (*e.g.* Trp, Leu, Lys) destabilizes the fold of the C-domain. Interestingly, substitution with negatively charged residue (Asp) also retains the overall fold.

Obtaining a full-length structure of SpaD had initially been a challenge due to its anisotropic nature and unresolved spots in the X-ray diffraction pattern. Later, usable X-ray diffraction data were collected from two different crystal forms (orthorhombic and hexagonal) of SpaD by optimizing the parameters in crystallization and diffraction experiments. The discovered full-length structures of SpaD displayed three immunoglobulin-like domains (N, M and C) with two different conformations referred as linear (open) and bent (open) states (Fig. 10). Interestingly, the N-domain showed the presence of an uncommon YPKD pilin motif with an intact isopeptide bond between lysine and aspartate. The primary structural analysis showed that this feature is mainly conserved among *Lactobacillus* strains. The C-terminal tail containing the sorting motif projects from the C-domain. The full-length structure of the open state revealed remarkable structural differences from that

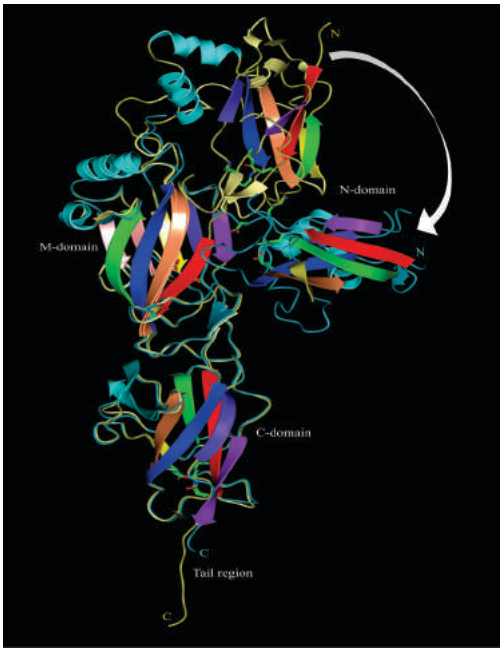
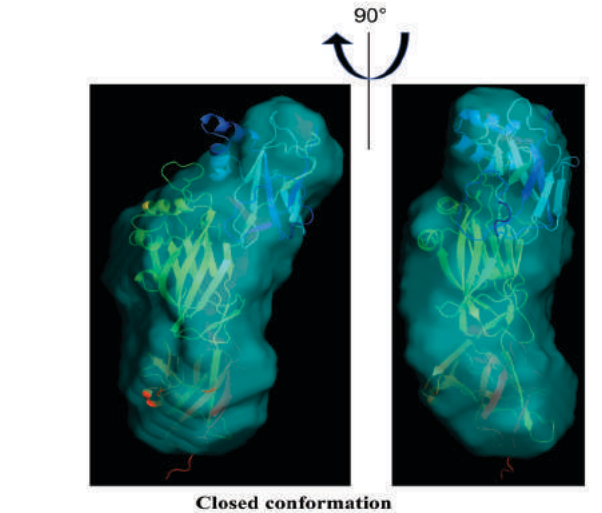


Figure 10: Crystal structure of SpaD in the open (bent) and closed (linear) conformational states (superimposed). Rainbow style color indicates core of the three domains (N, M, and C), sticks represents residues involved in isopeptide bonds, and the black arrow shows N-domain movement. Loop regions are in gold and cyan for linear and bent conformation respectively.



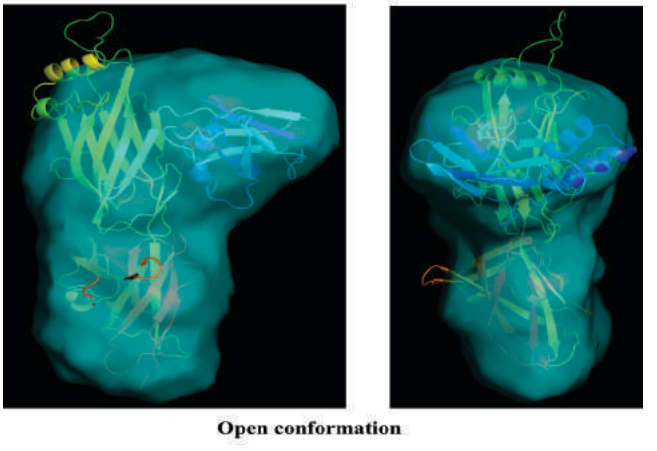


Figure 11: SAXS analysis of SpaD. Two orthogonal views of the overlap of the SAXS envelope (cyan) and the best-fitted crystal structure model (ribbon representation) for the closed (top) and open (bottom) conformational states. The ribbon representation of the crystal structure model is in blend through colors, starting with blue at the N-terminal and ending with red at the C-terminal.

of the closed state, specifically in the N-domain. The N-domain is rotated and positioned perpendicularly next to the M-domain instead of a linear arrangement as seen in the closed state (Fig. 10). In the open state, the N-domain does not form an isopeptide bond due to conformational changes. Such a bent conformation had never been observed previously for any sortase substrates (pilins) including pathogenic strains. This bent conformation appears to be an intermediary state, which is necessary to facilitate the sortase-mediated pilus assembly. Hence, the linear and bent conformations likely represent two different snapshots in the pilus assembly. The structural analysis by small-angle X-ray scattering (SAXS) (Fig. 11), domain motion analysis, and molecular dynamic simulation have also supported the two possible states.

The sequence and structural comparisons, and examination of molecular packing in the crystals of SpaD have brought new mechanistic insights for sortase-mediated pilus assembly that has been coined the *expose-ligate-seal* mechanism. According to this mechanism, the mobile loop of the flexible N-domain in the open state unfolds to leave the linking-lysine unprotected for favoring nucleophilic attack (*Expose*). This leads to C-type sortase-mediated ligation between the N- and C-domains of two adjoining pilins *i.e.* between C-terminal LPXT-motif threonine and N-terminal pilin motif linking lysine (*Ligate*). Then, the mobile loop in the N-domain undergoes a conformational change and becomes ordered by engaging in stabilizing interactions with the adjoining C-domain. This process encloses the groove-like hydrophobic pocket in the N-domain (*Seal*). The intra domain isopeptide bond formation in the N-domain likely takes place at the end to further support the sealing like a guy-wire for a utility pole. The proposed mechanism explains how sortase-mediated pilus assembly takes place at the bacterial cell-surface through covalent docking of backbone pilins. This study further explained why the N-domain is absent or flexible in the known pilin structures. The structural data from this project have been made available to the public by deposition in the Protein Data Bank (PDB) with IDs 5YU5, 5YXO, 5YXG, 5Z0Z and 5Z24 (Chaurasia *et al.*, *Comms Bio,* 2018).

The initial structural model of tip minor pilin SpaC, which is primarily responsible for SpaCBA pili-mediated adhesion has revealed the presence of vWFA (von Willebrand factor type A) domain with MIDAS (metal ion-dependent adhesion site) and CnaB (Collagen-binding adhesin B repeat fold) domains at N- and C-terminal regions respectively. Truncated forms of vWFA and CnaB domains have been recombinantly produced. These purified fragments are being used to study their functional and

structural role. Similarly, structure determination of basal pilin SpaE has also been completed. It reveals a two-domain architecture with a CnaB fold. The structural analysis is under progress.

Lactobacillus ruminis is another strain which was initially isolated from humans and subsequently from cattle, pigs, and birds. *L. ruminis* has been described as an autochthonous (i.e. indigenous or original inhabitants) microbiota present in the gastrointestinal tract (GIT). In addition to its probiotic effects (immunomodulation, inhibition of pathogens, and maintenance of gut flora), *L. ruminis* is an indispensable agent in fermentation of foods and feed. Its genome contains pilus operon (*lrpCBA*), which encodes three pilins (LrpA, LrpB and LrpC) and one sortase (SrtC). The LrpCBA pilus type appears to be different from the SpaCBA and SpaFED pili of *L. rhamnosus* GG, and likely represents a third sortase-mediated pilus type in *Lactobacillus* species. In contrast to SpaCBA and SpaFED pili, the LrpCBA lacks mucus-binding abilities 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. Towards understanding the colonization strategy of *L. ruminis,* and how it differs from *L. rhamnosus* GG and pathogenic strains by structural investigation, the purification strategy has been optimized to recombinantly produce pilus components of LrpCBA pilus at a large-scale.

The oral cavity harbors the second most abundant microbiota after the GIT. This microbial community plays 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 such as carries and gingivitis. The streptococci bacteria colonize the oral tissues immediately after brushing within few minutes for plaque formation. These species have become a focus for investigation as they are the primary colonizers in plaque formation and also providing adhesion sites for secondary colonizers during oral biofilm development. Pilus components from these primary colonizers are being produced for structural and functional characterization towards understanding and targeting pili-mediated interaction to maintain health and control diseases. Like bacteria, viruses also utilize their surface components known as spikes to mediate attachment and interact with the host. Studies on structural proteins from Chikungunya virus (CHIKV) are being carried out to elucidate mechanism of virus attachment and interactions as part of collaborative project for facilitating development of antiviral treatments. In the future, structural investigation programme on microbial molecules and assemblies that mediate interaction with host surfaces will be continued towards the projected goals.

Role of platelet and leukocytes in the pathogenesis of thrombosis and inflammations under various diseases and stress conditions

Dr. Prasenjit Guchhait

Principal Investigator

In order to understand the complex pathophysiology of clinical problems such as thrombosis and inflammations in various diseases, we are investigating, how a pathogenic factor of thrombosis is increasing the risk of inflammation, and vice versa, in patients? We are investigating the above clinical complications in diseases including 1) hemolytic disorders such as PNH and sickle cell disease; 2) viral infections such as Dengue and Japanese encephalitis; 3) hypoxia-induced thrombosis and edema in brain of the mountain travelers from low lands.

Co-Investigators

Tulika Seth, AIIMS, New Delhi
Naval K. Vikram, AIIMS, New Delhi
Gopal Patidar, AIIMS, New Delhi
Renu Saxena, AIIMS, New Delhi
Parvaiz Kaul, SKIMS, Srinagar
Ghulam Mohamad, SNM Hospital, Leh
Subodh Kumar, AIIMS, New Delhi
JC Suri, Safdarjang Hospital, New Delhi,
M.K. Sen, Safdarjang Hospital, New Delhi
Shinjini Bhatnagar, THSTI
Sudhanshu Vrati, RCB
S.V. Eswaran, RCB
Perumal Thigarajan, Baylor College of Medicine, USA
Josef T Prchal, University of Utah, USA
Jing-fei Dong, University of Washington, USA
Navaneetha Rao, RythRx Therapeutics, USA

Group members

Teena Bhakuni
Rashi Singhal
Amrita Ojha
Angika Bhasym
Sulagna Bhattacharya
Nishith Shrimali
Saibal Saha
Jaya Saini

Our laboratory is focused on elucidating the role of platelets and immune cells in regulating the pathogenesis of hyper-coagulation, thrombosis and inflammation in (1) hemolytic diseases such as sickle cell anemia and Paroxysmal Nocturnal Hemoglobinuria (PNH), (2) viral infections such as Dengue and Japanese encephalitis, and (3) high-altitude mountain hypoxia. Under this theme, we are investigating how:

1. Platelet activation induces inflammation and alters immune homeostasis in patients with hemolytic disorders such as sickle cell disease (SCD) and PNH.
2. Platelet activation leads to thrombocyto-penia in Dengue infection.
3. Platelet proteins promote rapid propagation of Dengue virus (DV) and Japanese encephalitis virus (JEV) during infections.
4. High-altitude mountain hypoxia differentially regulates the coagulation and inflammatory pathways, and alters immune responses among travelers from low lands.

Hemolytic disorders

Hemolytic disorders: In hemolytic disorders such as aplastic anemia, paroxysmal nocturnal hemoglobinuria (PNH), hemolytic uremic syndrome (HUS), thalassemia and sickle cell disease (SCD) the release of excessive free-hemoglobin (Hb) or Heme in plasma triggers

many cytotoxic effects. We have described that the free-Hb activates the circulating platelets potently and promotes pro-thrombotic as well as hypercoagulative states in hemolytic patients (Singhal *et al.*, *Hematologica*, 2015; Annarapu *et al.*, *PLOS One*, 2016, 2017). The activated platelets and their cytokines further stimulate the leukocytes including monocytes, macrophages and neutrophils, and regulate their functions. We have shown that after engulfing Hb-activated platelets from circulation the monocytes were transformed into highly pro-inflammatory states, and mediated the inflammatory complications in patients with PNH and SCD (Singhal *et al.*, *Clin Immunol,* 2017). Further our studies have shown that after engulfing Hb-activated platelets the monocytes were differentiated into pro-inflammatory M1 macrophages, whereas after engulfing only Hb monocytes were differentiated into anti-inflammatory M2 macrophages (Fig. 12; Singhal *et al.*, *Eur Jr Immunol,* 2018). We also observed that after engulfing Hb-activated platelets the neutrophils were highly activated and secreted pro-inflammatory cytokines and enzymes such as MPO and elastase in hemolytic conditions in PNH patients and mice model with intravascular hemolysis (Bhasym *et al.* 2018, under communication). Our focus is to investigate the mechanism, how Hb along with secretome of Hb-activated platelets are involved in the development of pathophysiological conditions such as chronic and systemic inflammatory conditions in hemolytic patients including PNH and SCD.

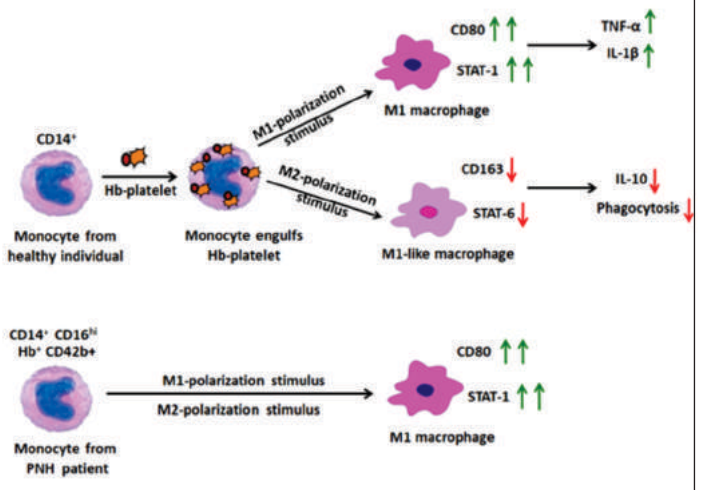


Figure 12: Hb-activated platelets differentiate monocytes into pro-inflammatory (M1) macrophages. *Monocytes isolated from healthy individuals engulf Hb-activated platelets in culture and in the presence of M1 polarization stimulus (GM-CSF+IFNγ+LPS) differentiates into M1 macrophages expressing high levels of CD80 and STAT-1 and releasing high amount of pro-inflammatory cytokines such as TNF-α and IL-1β. Even in the presence of M2 polarization stimulus (M-CSF+IL-4+Il-13), Hb-activated platelets have the tendency to skew these macrophages to M1-like macrophage with decreased levels of CD163, STAT-6 and IL-10 and reduced phagocytic capacity. Monocytes from PNH patients differentiates into pro-inflammatory (M1) macrophages independent of M1 or M2 polarization stimulus.*

Viral infections

In a recent work, we have shown that the binding of Dengue virus (DV) activates platelets, and platelet activation states determine the severity of platelet clearance/destruction, which is one of the causes of thrombocytopenia in DV patients (Ojha *et al.*, *Sci Rep,* 2017). While investigating the above mechanism, we observed that the monocytes when engulfed the DV-activated platelets, displayed unique phenomenon. The replication of DV was increased 4-times more in monocytes that engulfed DV-activated platelets than only DV of same MOI (Fig. 13). The similar elevation in DV replication was observed when the monocytes were treated with the supernatant of DV-activated platelets. With help of proteomic analysis of above monocytes lysate we observed that the host protein platelet factor 4 was associated with DV replication. We confirmed the enhanced DV replication in presence of rhPF4. The anti-PF4 antibody or the antagonist to CXCR3 (PF4 receptor), namely AMG487 significantly inhibited the DV replication as well as propagation. Further we observed that above same mechanism of PF4-mediated elevation in replication of JEV in monocytes and microglia cells. Furthermore, the AMG487 injection to JEV infected mice decreased significantly the infection and increased the mice survival (Ojha *et al.* 2018, under communication).

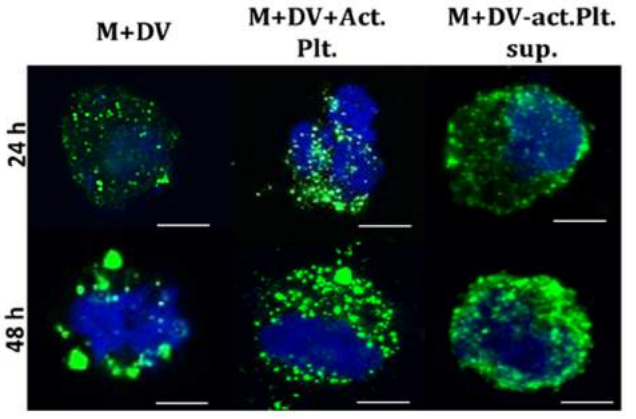


Figure 13: Elevated replication of DV in monocytes in presence of intracellular platelets: *Primary monocytes were incubated with DENV type-2 (DV2) alone (MOI=3) or DV2-activated platelets or DV2-activated platelets supernatant. The DV2 replication was measured using intracellular staining with anti-dsRNA antibody (AlexaFluor-488/Green) at 24hr and 48hr of post-infection using confocal microscopy. Nuclear DNA was stained with DAPI (Blue). Increased expression of dsRNA was observed in monocytes, which have engulfed DV2-activated platelets when compared with DV2 alone. Scale bar = 5μm.*

High-altitude hypoxia

High-altitude hypoxia increases the risk of edema and intravascular clot formation among the travelers from sea levels. However, the native highlanders including Tibetans live normally under extreme hypoxic condition (even 40% less pO_2 than sea-level) at 3500-meters altitude. We have shown that the novel mutations in *EGLN1* gene, which encodes Prolyl Hydroxylase-2 (PHD2, the negative regulator of hypoxia inducible factor (HIF)-1α, which is the master sensor of oxygen in the human body), supports the adaptation of native Tibetans in the high mountains (Lorenzo *et al.*, *Nat. Genet.*, 2014, Tashi *et al.*, *J Mol Med,* 2017). Currently we are studying the factors associated with (a) coagulation and thrombotic complications, (b) inflammations and edema, and (c) innate and adaptive immune functions among the native Tibetans with and without above $PHD2^{D4E/C127S}$ mutations from high altitudes in Kashmir Valley (2000-3500 meter altitudes) and sea levels (Delhi, India). We observed that the monocytes from Tibetans with $PHD2^{D4E/C127S}$ exhibited diminished secretion of pro-inflammatory cytokines including IL-1β, TNF-α, IL-6 and tissue factor in response to ligands, compared to wild type counterparts (Bhattacharya *et al.* 2018; World Symposium on Mountain Medicine). Our investigation is focused on understanding whether the Tibetans with $PHD2^{D4E/C127S}$ are adapted against hypoxia-induced thromboinflammatory complications.

We will now investigate whether the Tibetans with $PHD2^{D4E/C127S}$ variants have an adaptive advantage against hypoxia-induced thrombosis and edema and delineate whether these $PHD2^{D4E/C127S}$ individuals have altered innate responses to infections. We will also investigate the advantage and disadvantage of the above mutations in Tibetans. The final goal is to identify targets that can protect the travelers from episodes of mountain sickness.

Virus Discovery and Molecular Understanding of Viral Functions

Dr. Kanchan Bhardwaj

Principal Investigator

Co-Investigator

Dr. Sudhanshu Vrati

Group members

Himani Sharma

There are more microbes (bacteria and viruses) than the human cells living within an individual. Scientists have analyzed human fecal samples from different parts of the world to learn about the viruses that live in the human gut. They have found that there are useful viruses in the human gut and that more can be learnt about gut viruses by investigating fecal samples from people with different lifestyles. We are studying the viruses that reside in the gut of Indian people. Knowledge gathered through this work would be useful for learning more about viruses and may help find a cure for some of the diseases related to the gut.

Viruses, the most abundant microbes on the planet, are obligate parasites and infect all forms of life. They interact with their hosts in various ecosystems, living (e.g. human body) and non-living (e.g. oceans) and play important roles in maintenance of the ecosystem. They cause diseases, impact functioning/evolution of their host and are useful as biotechnological tools. Viruses and the genetic complexity that they bring are being discovered continuously.

Metagenomic studies have identified several known and novel viruses at various body sites of healthy individuals. More than 100 million virus-like particles are estimated to be present per gram of human feces, representing the gut virome. Emerging evidences support the idea that gut viruses, including bacteriophages and eukaryotic viruses play important roles in human health and physiology. Therefore, a comprehensive understanding of the gut virome is of interest for its clinical implications. In addition, investigation of the gut virome has the potential of discovering novel phages, as gut is one of the richest concentration reservoirs of phages.

For the future translational applications, there is a need for independent characterization of the gut viromes in unique populations. Our aim is to create an in-depth inventory of the viruses that are found in the gut of healthy Indian adults, describe their functional repertoire, dynamics, extent of inter-individual variability and temporal stability. Our approach is to investigate the gut virome through metagenome sequence analysis of fecal virus like particles (VLPs) and total fecal DNA, collected longitudinally over one year at six months' interval. We will analyze samples from individuals between the ages of 20 and 35 years, who have normal body mass index, normal bowel frequency, no history of chronic intestinal disease or autoimmunity, had

balanced meals at regular intervals and have not received antibiotics in the 6 months before sampling.

Towards the above goal, we have obtained written consent from volunteers for the collection of samples at three time points. Samples have been collected for the first time point and stored at -80°C until their analysis is completed. To be able to sequence viral genomes, virus-like particles (VLPs) need to be purified first. In earlier analyses of the gut virome, VLPs were purified by cesium chloride (CsCl) density ultracentrifugation. The yield of DNA extracted from VLPs in these studies was very low and required multiple displacement amplification (MDA) method to amplify VLP DNA with random primers and Phi 29 DNA polymerase before sequencing. Both the use of CsCl and genome amplification before sequencing could introduce bias. CsCl solutions at the densities used to band viruses are hyper-osmotic and some of the viruses are sensitive to CsCl, leading to an under-representation of sensitive viruses in the analyzed population. Further, DNA amplification by MDA

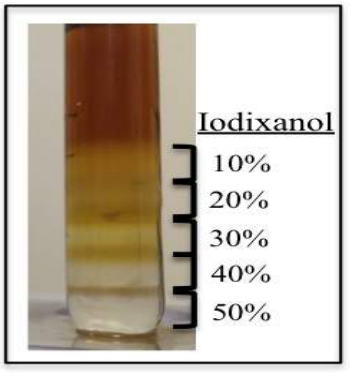


Figure 14: Purification of fecal VLPs through density gradient ultracentrifugation. *Fecal samples were resuspended in buffer and homogenized by vortexing in presence of glass beads. Extracts were centrifuged and the supernatants were filtered through 0.45 µm membrane syringe filters. The filtrates were loaded on density gradients of Iodixanol (10%-50%). Gradients were centrifuged at 200,000 X g for 2 h at 4 °C. % iodixanol is indicated.*

leads to a preferential amplification of small ssDNA viruses, leading to their over-representation in the analyzed population. For an unbiased analysis of viral diversity, we optimized the amount of starting material required to obtain sufficient nucleic acid recovery. We have also optimized purification of VLPs through a density gradient that is prepared with OptiPrep (Fig. 14). OptiPrep is a solution of 60%

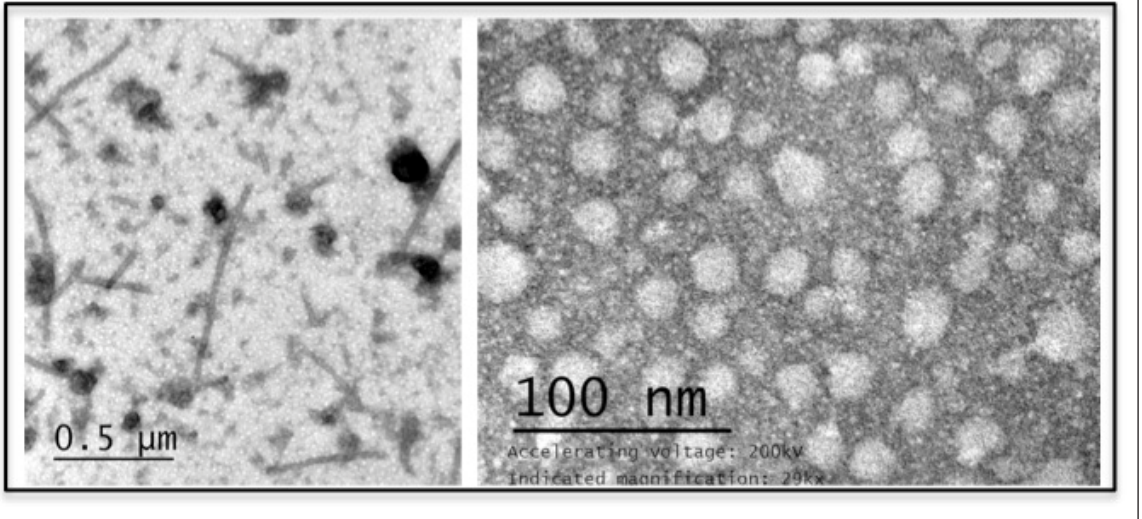


Figure 15: Electron micrographs of the purified VLPs. *Purified virus particles were adsorbed on carbon-coated grids followed by staining with phosphotungstic acid and images were captured on transmission electron microscope. Scale bar is indicated.*

iodixanol in water. Iodixanol solution is not only non-toxic, non-ionic and can be made iso-osmotic at all useful densities but has also been shown to retain virus infectivity at least 100-fold as compared to CsCl gradients. We confirmed the recovery and purification of VLPs by transmission electron microscopy

and extracted DNA for sequencing (Fig. 15). We have also extracted bacterial DNA directly from fecal samples, following published protocols.

We will use Illumina HiSeq2500 system for sequencing the extracted VLP DNA as well as bacterial DNA. After obtaining the data, we will annotate and classify the viral sequences based on their similarity to the available reference genome databases. We will partition them into eukaryotic viruses or bacteriophages of known or novel categories. To estimate inter-individual variability, we will use statistical methods and calculate alpha-diversity among at least ten samples. Similarly, statistical methods will also be used to calculate beta-diversity, which will indicate temporal stability of the virome. We will determine viral dynamics through the determination of bacteria-to-virus ratio in fecal samples as well as perform bioinformatics analyses of the sequences obtained from fecal virus like particles (VLPs) in tandem with bacterial DNA in the corresponding sample. Lastly, we will define functions encoded by the gut viruses in Indian adults through bioinformatics analysis.

This study will lay a baseline to start analyzing how the gut virome may be altered under clinical conditions that are associated with gut microbiome dysbiosis and identify biomarkers. It will also provide important details for building a rational approach to develop therapeutics for conditions that are associated with gut microbiome dysbiosis. In earlier studies, significant differences in the gut microbiome and gene repertoires have been noted between residents of distinct geographic locations. Through this study, we expect to reveal characteristic features such as the viral diversity, dynamics and functionality encoded by the gut viruses in healthy Indian individuals. It will allow us to identify features that are unique to Indian individuals and features that it shares with other populations. Identification of similarities and uniqueness among ethnic groups will be helpful in starting to understand the correlation between the gut virome and factors such as host genetics, early microbial exposure and environment. Novel viruses recovered from the gut of Indian individuals could be investigated for their potential as biological tools in addition to understanding their role in human health. Describing the genomes of recovered novel viruses and viral proteins will provide important information on the viral life cycle and their interaction with the host. Novel viral proteins could also have clinical implications and use as research reagents. Identification of unexpected viruses may affect the way we view their impact on human health. For instance, picobirnaviruses were indicated in the pathogenesis of gastroenteritis and diarrhea but their subsequent discovery in healthy infants has left the pathogenic capability of picobirnaviruses in doubt. Sequencing data will be useful for the assessment of variations and evolution of viruses.

Biology of medically important viruses

Sudhanshu Vrati

Program Coordinator

Principal Investigators

Deepak T. Nair
Sivaram V.S. Mylavarapu
Deepti Jain
Vengadesan Krishnan

Group members

Shivendra Singh
Amar Prajapati
Kuldeep Verma
Pankaj Kumar Sahoo
Amit Yadav
S. Chandru

Infectious diseases pose an ever-increasing threat to the well-being of the 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 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. We are aiming to identify potential antiviral molecules by understanding the structure and function of the important viral proteins. To this end we aim to express and purify the E1, E2, NSP1 and nsP4 proteins of CHIKV and study their biology. We will use *in silico* or *in vitro* methods to then identify small molecules that may interfere with a critical biological function of the protein thereby inhibiting the virus infectivity. Progress of the various projects under this program is reported below.

Generation of a recombinant CHIKV expressing GFP

A full-length of infectious clone of the La Réunion island isolate of CHIKV (LR2006 OPY1 strain) has been made by cloning the cDNA in pSinRep5 vector. This clone has an SP6 promoter at the beginning of the viral genome for *in vitro* transcription and a Not I linearization site immediately following the polyA$_{40}$ tail at the 3'-end of the viral cDNA. In this clone, GFP is expressed 5' to the structural genes by placing the GFP coding sequence under a second subgenomic promoter. During viral replication, subgenomic RNA transcribes from genomic RNA and serves as the template for protein synthesis. The recombinant virus thus produced is capable of expressing GFP (Fig. 16) and thus could be used for high throughput screen of antivirals.

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

The aim here is to study the interaction between the CHIKV structural envelope protein (E1 and E2) and their host receptors at the atomic level to elucidate mechanism(s) of CHIKV envelope proteins mediated attachment for facilitating development of antiviral treatments. Structural models for CHIKV envelop proteins (E1 and E2) and their predicted interacting receptors were retrieved from the PDB (Protein Data Bank). The

protein structural models were energy minimized to correct any structural errors. Then, the docking and *in silico* experiments were carried out using the energy minimized structural models. For initial docking, online tools such as ClusPro were used. Representative poses from the top ranked clusters were chosen for further structural analysis. The hotspots comprising key surface residues involved in the interaction were identified by the structural analysis through visualization, buried surface area calculation and analyzing the key interactions (electrostatic, hydrophobic and van der Waals interactions). Validation of these results by experiments and screening of a library of compounds that could disrupt the key interactions are in progress.

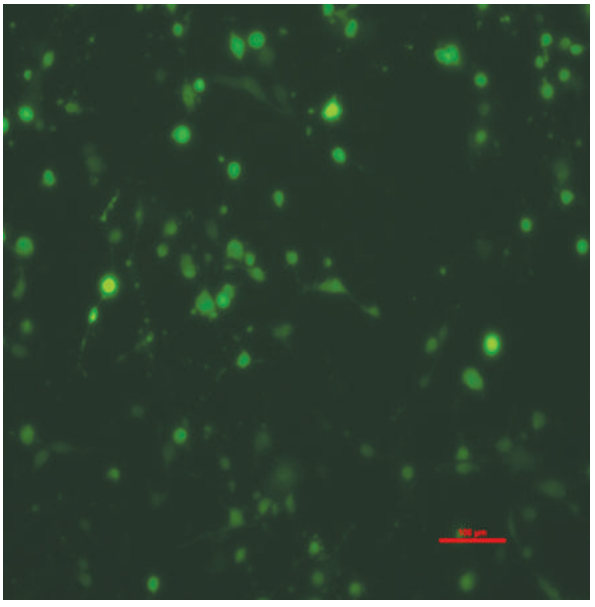


Figure 16: *BHK cells infected with recombinant CHIKV expressing GFP*

For experimental validation, attempts were made to produce E1 and E2 proteins by recombinant expression in *E. coli* with His-tags, but the produced proteins were insoluble. Subsequently, expression with a GST-tag and codon-optimization were explored to increase the solubility. Despite intensive expression optimization, the expressed proteins were found to be in inclusion bodies. Hence, expression in other systems, tags, and making constructs for individual domains of E2 have been planned.

Studies on CHIKV nsP4 protein and the replication complex

The nsP4 protein of Chikungunya virus houses a RNA-dependent-RNA polymerase (RdRP) activity and is centrally involved in replication of the RNA genome of this Alphavirus. A structural snapshot of the nsP4 protein bound to substrate RNA and incoming NTP can be exploited to identify small molecules that can perturb nsP4 function and serve as lead molecules for the development of novel drugs against the debilitating disease caused by this virus. To carry out structural and biochemical studies on CHIKV nsP4, we obtained a codon-optimized synthetic construct of the full-length enzyme. The full length (residues 1-611) and different truncated constructs of the nsP4 protein were cloned into two different expression vectors that will yield fusion proteins with His- and GST- tags. All the constructs tested did not yield soluble protein and the expressed protein entered in the pellet fraction due to aggregation. For the constructs tagged with His-tag, different refolding protocols in the presence of various additives were tested to obtain soluble protein. For one of the constructs, a protocol involving rapid dilution of the denaturant provided microgram quantities of protein that eluted in the bed volume of a gel filtration column. However, the protein was not active and this may be due to incorrect folding. At present, the constructs are being re-cloned into new expression vectors that will give rise to fusion protein with SUMO- and MBP- tags. In parallel with the efforts to obtain soluble protein, we have utilized bioinformatics tools to generate a computational model of the RdRP region of nsP4 (residues 285-577) in complex with substrate RNA and incoming CTP. The model was built using the co-ordinates of the functional complexes of the RdRPs from Foot & Mouth Disease Virus and the Norwalk Virus. The computational model was subjected to energy minimization and validation tests. The model (Fig. 17) is currently being analyzed to identify interfaces that can be targeted for disruption by small molecules. *In silico* screening to discover potential binders that will interact with the identified surfaces will be initiated soon. The small molecules identified in this manner will be assessed for their ability to perturb nsP4 function using biochemical and cell-based virus replication assays.

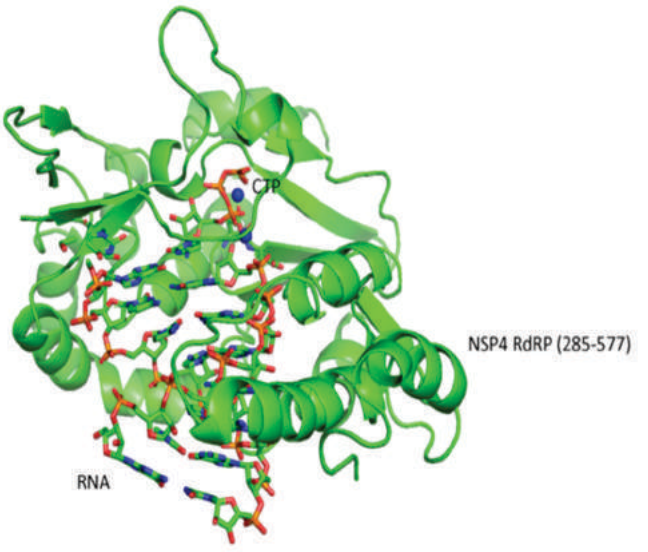


Figure 17: *Computational model of CHIK RdRp in complex with RNA and CTP.*

To define the CHIKV replication complex, we are also studying the components of the viral and host proteins interacting with nsP4 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 U2OS (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. Using TAP-tagging of nsP4 followed by affinity purification and mass spectrometric analyses, we have successfully identified CHIKV-nsP4 interacting cellular host proteins and narrowed down to a small list of nine high confidence and reproducible interactors. These novel host proteins are likely to play important but as yet uncharacterized roles in the biology of the virus inside host cells. These interactions will be validated in CHIKV-infected cells using different methods.

Studies on CHIKV nsP1 protein

nsP1 is the primary enzyme containing methyltransferase and guanylyltransferase activity and is involved in RNA capping in CHIKV. The nsP1 adds a 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 the N-7 atom of GTP, covalent attachment of m7-GMP to nsP1 followed by transfer of this adduct to the 5' end of viral RNA. The project involves structure function analysis of nsP1, which is a potential target for anti-viral therapy. We have standardized the expression and purification of the full length nsP1 using different constructs and expression hosts. The purified enzyme is active. Efforts are underway to convert the assay into a high throughput screen for screening inhibitors. Site directed mutants will be prepared to identify residues that are essential for the methyltransferase activity of the enzyme. The purified enzyme is prone to degradation. An *ab initio* structural model for nsP1 has been built and will be utilized for designing the constructs that are more stable and suitable for structural studies.

Mechanisms of proteostasis and disease regulation

Dr. Tushar Kanti Maiti

Principal Investigator

Co-Investigator

Neel Sarovar Bhavesh, ICGEB, New Delhi

Group members

Sanjay Kumar
Raniki Kumari
Manisha Kumari
Sandhini Saha
Amit Kumar Dey
Bhoj Kumar
Roshan Kumar
Abhishek Kumar Singh

Parkinson's disease (PD), one of the most common nervous system disorders, affects elderly populations worldwide and is accompanied by shaking, rigidity, slowness of movement, difficulty with walking and ultimately death. Genetic factors, aging and excessive exposure to environmental toxins contribute to the etiology of PD. The pathological hallmark of PD is the irreversible deposition of proteinaceous aggregates in the midbrain leading to loss of dopaminergic neurons. PD is considered as a protein misfolding disorder and therapeutic intervention against PD is still limited. Our lab aims to understand the mechanism of protein aggregation and neurotoxicity, which will pave the way for designing novel therapeutic molecules. Our long-term goal is to develop small molecule inhibitors that prevent aggregation of proteins involved in PD.

Protein metabolism is essential for normal cellular function and it involves synthesis, folding, transport and degradation of proteins. Dysregulation of protein quality control leads to various diseases including cancer and neurodegeneration. The aim of our research is to delineate the mechanism of protein quality control systems in these diseases.

Deubiquitinating enzymes and disease regulation

Human genome analysis and proteomics data reveal almost one hundred deubiquitinating enzymes (DUBs), which majorly regulate protein homeostasis in cells. However, molecular functions of most of the DUBs are not well elucidated. Furthermore, functional loss of deubiquitinating enzymes leads to many diseases including cancer, cardiovascular and neurodegenerative diseases. Our aim is to understand the possible molecular mechanisms underlying these diseases.

During 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 cellular fate, cell survival and genome integrity. Cylindromatosis protein (CYLD), a tumor suppressor protein and USP family deubiquitinating enzyme, specifically cleaves Lysine-63 linked polyubiquitin chains from its substrates. CYLD regulates diverse cellular processes ranging from cell cycle progression to inflammation. Recently, we have demonstrated that CYLD cancer-associated mutants show structural instability, impaired ubiquitin binding and loss of

catalytic activity. The diminished deubiquitinase function triggers tumor progression due to enhanced activity of inflammatory and cellular proliferation molecules, including NF-kB, c-jun, cyclin-D1, p38, Erk (1/2) Map kinases. Overall, our results unambiguously demonstrate that structural destabilization and subsequent aggregation abrogate its cellular mechanism leading to adverse outcomes (Johari and Maiti, 2018, *Biochim. Biophys. Acta*).

The mass spectrometry-based quantitative proteomics analysis of CYLD knock out (KO) SH-SY5Y cells displays a down-regulation in apoptosis and up-regulation in cell cycle pathways, ER stress response and autophagy, which are major cellular processes. It has been demonstrated that loss of CYLD inhibits apoptosis via constitutive activation of NF-kB mediated expression of pro-survival genes. The increase in the cellular chaperone pool, attenuation of the translational machinery, blockage of ER-Golgi trafficking and a rise in cellular stress sensor proteins levels in CYLD knockout cancer cells have also been observed in our mass spectrometry data. Cellular experiments have also been carried out to corroborate the mass spectrometry data. CYLD depletion leads to an increase in misfolded protein load in cells that eventually induces the unfolded protein response (UPR) pathways. UPR regulates multiple signals that balance cell death and survival during stress. An efficient way of accomplishing these signals is the integration of Autophagy within the UPR signaling network. Our data demonstrated a novel mechanism by which CYLD can curb ER stress and autophagy that could underlie critical dysfunctions in both cancer and neurodegenerative diseases (Fig. 18).

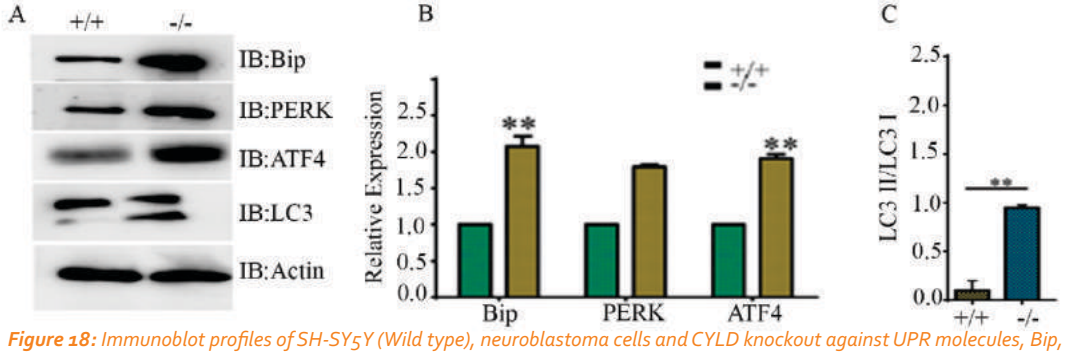


Figure 18: *Immunoblot profiles of SH-SY5Y (Wild type), neuroblastoma cells and CYLD knockout against UPR molecules, Bip, PERK, ATF4 and LC3-I to LC3-II conversion suggested that loss of CYLD causes adequate ER stress to activate different UPR arms and autophagy in vitro.*

Protein aggregation mechanism in neurodegenerative diseases

Neurodegenerative diseases are characterized by progressive loss of structure and function of neurons in specific regions of the brain. The hallmark of pathogenesis of these diseases is often associated with the abnormal accumulation of intracellular or extracellular protein aggregates that are characteristic for each disease. The impairment of protein quality control leads to the abnormal accumulation of disease-specific proteins. Here we aim to understand the mechanism of protein aggregation and its toxicity that contribute to disease outcomes.

Parkinson's disease (PD) is the second most common neurodegenerative disorder, which is characterized by the death of dopaminergic neurons in the substantia nigra pars compacta region of midbrain and formation of intracellular eosinophilic proteinaceous aggregates, termed as Lewy bodies (LB). The LB in the brain is a hallmark of PD with α-synuclein as its major component. α-Synuclein is deposited in LB in hyperphosphorylated (Ser-129) form with β-sheet-rich fibrillar structure. Besides α-synuclein, LRRK2, Parkin, PINK1 and DJ1 proteins are also present in LB and are well characterized. A

recent study has shown that more than 500 proteins are present in LB and among them, 40 proteins are co-enriched with α-synuclein. Genetic mutations like A53T and H50Q, gene duplication, and post-translational modification like nitration and phosphorylation induce α-synuclein to form oligomers and higher order aggregates. Recently, we have shown that A29S and A18T mutants of α-synuclein induce its increased fibrillation and cytotoxicity (Kumar *et al*, 2018, *ACS Chem. Neurosci.*). We have also shown that S-nitrosylation of UCHL1 induces structural destabilization and promotes α-synuclein aggregation (Kumar *et al,* 2017, *Sci. Rep.*). α-Synuclein produces various pathogenic oligomeric species in Parkinson's disease conditions and these oligomeric species secrete out from the cells. The extracellular α-synuclein oligomers are taken by the cells via different methods. Extracellular α-synuclein interacts with the cellular membrane and impairs the membrane structure and forms membrane pores. The α-synuclein membrane interaction and pore-like structure have been shown in the model membrane. Prior to our study, there was no evidence reported of the nanoscopic view of cell membrane damage and downstream signaling events induced due to membrane perturbations by α-synuclein. We have demonstrated membrane damage and pore formation by α-synuclein using atomic force microscopy (Fig. 19). There is altered ionic homeostasis due to membrane damage, which induces enormous nitric oxide generation. The nitric oxide post-translationally modifies cysteine residue of actin, Parkin, Hsp70, UCHL1 and GAPDH, which promotes apoptotic cell death (Kumar *et al.*, 2018, *Biomacromolecules*).

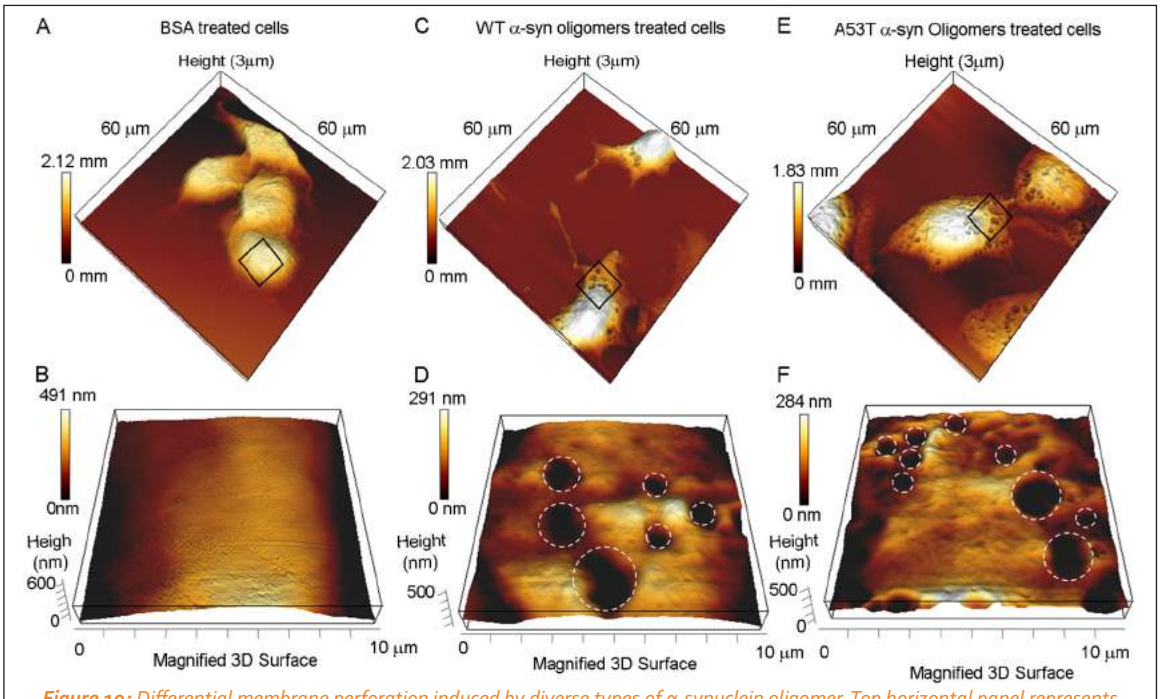


Figure 19: *Differential membrane perforation induced by diverse types of α-synuclein oligomer. Top horizontal panel represents 3D atomic force microscopic images (60μm x 60μm) of SH-SY5Y cells. Lower panel represents magnified 3D surface (10 μm x 10 μm). (A, B) BSA (C, D) wild type α-synuclein oligomer (E, F) A53T α-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 SH-SY5Y cells treated with wild type and A53T α-synuclein oligomer but were absent in BSA treated cells.*

The cellular system is devised with various protective mechanisms to counteract the misfolded protein load. The ubiquitin-proteasome system, lysosomal degradation pathways and autophagy are the molecular machineries that evacuate the misfolded protein load and various chaperones play a critical role in protein refolding to attain proper conformation. However, breach in any of these protective mechanism leads to diverse diseases. We have investigated the molecular aspects of redox-sensitive chaperone DJ-1 towards α-synuclein aggregation inhibition and toxicity. Partially oxidized DJ-1

possesses an adhesive surface compared to oxidized and hyper oxidized DJ-1. The partially oxidized DJ-1 sequesters α-synuclein monomers and blocks the early stages of α-synuclein aggregation by suppressing the formation of α-synuclein nuclei. It also restricts the elongation of α-synuclein fibrils. We have demonstrated that partially oxidized form of DJ-1 remodels the mature α-synuclein fibrils by strong interaction and generates heterogeneous toxic oligomeric species. The fibril-derived oligomeric species disrupt membrane architecture, internalize and induce aberrant nitric oxide release in cells. Our results provide new insight into the molecular mechanisms by which the partially oxidized DJ-1 counteracts the initial stages of α-synuclein aggregation. Our study also reveals an amyloid fiber remodeling mechanism by a small chaperone (Fig. 20).

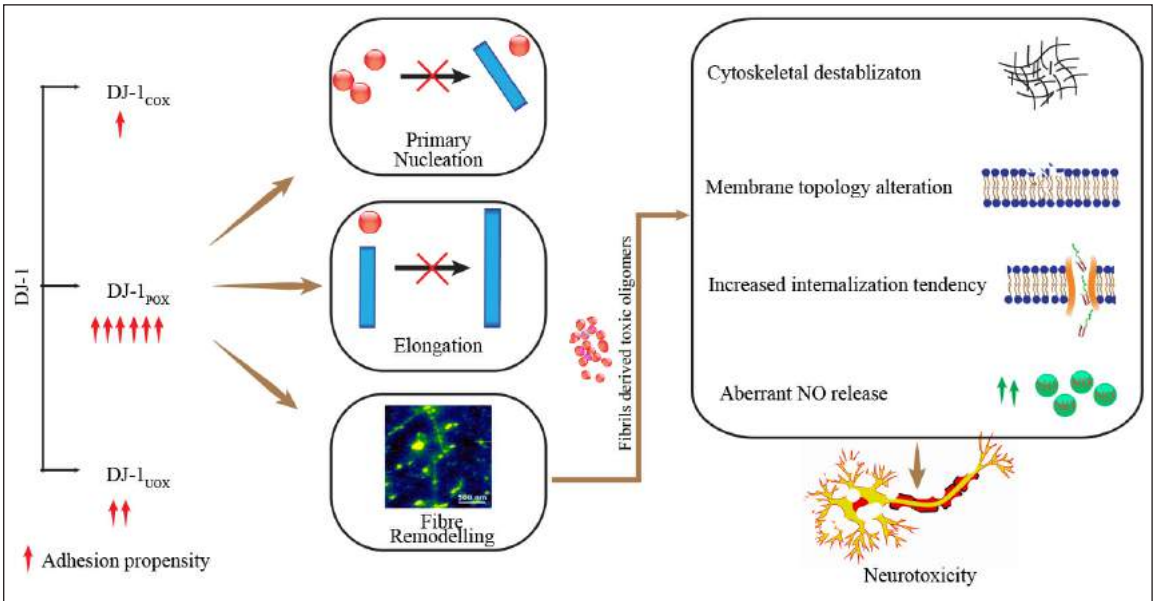


Figure 20: *Schematic showing the role of the partially oxidized form of DJ-1. DJ-1 in partially oxidized form possess strong surface adhesive property. The adhesive property of DJ-1 allows it to bind strongly to α-synuclein and inhibits primary nucleation and elongation of α-synuclein. Partially oxidized DJ-1 remodels mature α-synuclein fibrils to shorter species which induce cytoskeletal destabilization, alter membrane topology, and promote excessive nitric oxide production and neurotoxicity.*

Signals that Regulate Skeletal Muscle Structure and Function

Dr. Sam J. Mathew

Principal Investigator

Group members

Masum Saini
Bhargab Kalita
Megha Agarwal
Pankaj Kumar
Akashi Sharma
Shreyasi Das
Anushree

The skeletal muscle is an important tissue, crucial for mobility, posture, and support. Defective development of the skeletal muscle can lead to muscle diseases and cancer. One such is the childhood cancer rhabdomyosarcoma, where the cancer cells resemble muscle cells. Our work has identified the role played by two proteins in this cancer, which should lead to development of new therapies to treat rhabdomyosarcoma. Using animal models, we are also investigating proteins that help in muscle development and regeneration. Comprehending their function should help us understand muscle disorders and injury, thereby helping in effectively remedying them.

We are studying how cells become specialized during animal development to perform their specific functions in adults and how cells regenerate in response to injury or disease. Signals that regulate animal development and regeneration are also crucial in diseases such as cancer, which we are also interested in.

Our major goal is to understand the molecular mechanisms underlying skeletal muscle differentiation and how defective differentiation leads to skeletal muscle abnormalities and diseases, for which we have the following objectives: (1) Identify and characterize interactors of the MET receptor tyrosine kinase in Rhabdomyosarcoma (RMS), a cancer type where tumor cells exhibit characteristics of differentiating muscle cells, (2) Characterize the mutations in the skeletal muscle specific myosin isoform, Myosin Heavy Chain-embryonic (MyHC-emb) that lead to congenital contracture syndromes using *Drosophila* as model, and (3) Study the role of the skeletal muscle specific myosin isoform, Myosin Heavy Chain-embryonic (MyHC-emb) in mammalian skeletal muscle development and differentiation *in vivo* using a conditional knockout mouse model.

Rhabdomyosarcoma (Rhabdo=rod shaped; myo=muscle) or RMS is a predominantly pediatric soft tissue sarcoma, accounting for about 3% of all childhood cancers. Based on the tumor properties, there are two major sub-types of RMS: embryonal RMS (ERMS) and alveolar RMS (ARMS). RMS tumor cells exhibit characteristics of differentiated skeletal muscle cells and is thought to originate from the muscle stem cells. One pathway critical for proper skeletal muscle development is the receptor tyrosine kinase (RTK) signaling cascade, mediated by the MET receptor. MET is encoded by the *c-MET* proto-oncogene and MET levels have been reported to be dysregulated in RMS tumor cells derived from patients. Although some of the genetic lesions associated with RMS have been identified, the dysregulation of MET signaling in RMS has not been clearly understood.

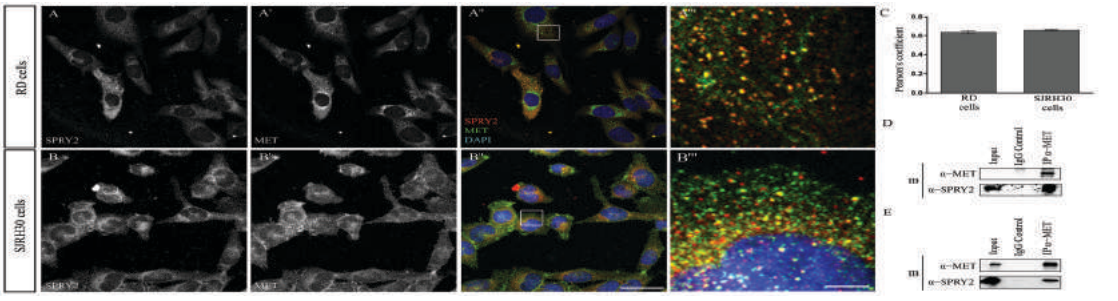


Figure 21: SPRY2 co-localizes and interacts with MET in RMS cells. *Immunocytochemical staining shows that SPRY2 (red) and MET (green) co-localize with each other in RD (**A** to **A"**) and SJRH30 (**B** to **B"**) cells, suggesting that SPRY2 might interact with MET in embryonal and alveolar subtypes of RMS. Panels **A"'** and **B"'** represent magnified images of regions marked by white squares in **A"** and **B"**. Nuclei are stained with DAPI (blue) and the scale bar is 50µm and 5µm in panels **B"** and **B"'**, respectively. Bar graph showing the mean Pearson's correlation coefficient for SPRY2-MET co-localization in RMS cells (**C**) (n=17), where perfect correlation = 1, no correlation = 0 and error bar represents ±SEM. Equal concentrations of cell lysates from RD (**D**) and SJRH30 (**E**) cells were immunoprecipitated (IP) using anti-MET antibody and immunoblotted with anti-MET and anti-SPRY2 antibodies. IP was also performed using IgG isotype control. SPRY2 immunoprecipitated with MET in both RMS cell lines (**D**, **E**).*

We found that similar to MET, levels of a bimodal regulator of receptor tyrosine kinases, SPRY2, are also upregulated in RMS. We found that SPRY2 regulates MET and silencing SPRY2 leads to MET downregulation in RMS. This led us to investigate whether MET and SPRY2 interact with each other. We found that MET and SPRY2 proteins co-localize with each other by immunofluorescence in both ARMS and ERMS cells (Fig. 21A-A"' and B-B"'). The co-localization was validated by a correlation using Pearson's coefficient (Figure 21C). To further confirm that MET and SPRY2 co-localization reflects a biochemical interaction between the two proteins, we performed co-immunoprecipitation using cell lysates from both ERMS (RD) and ARMS (SJRH30) cells. Notably, we found that MET and SPRY2 physically interact with each other in both ERMS and ARMS cells (Figure 21D, E).

MET has been reported to be important for RMS cell metastatic potential. In a transwell migration assay, we found that the percentage migration in SPRY2 or MET depleted ERMS cells was significantly impaired compared to controls (Fig. 22A, B). Next, we assessed the effect of silencing SPRY2 or MET on the clonogenic potential of RMS cells by anchorage-independent and -dependent colony forming assays. We found that silencing MET or SPRY2 significantly inhibited attachment independent clonal

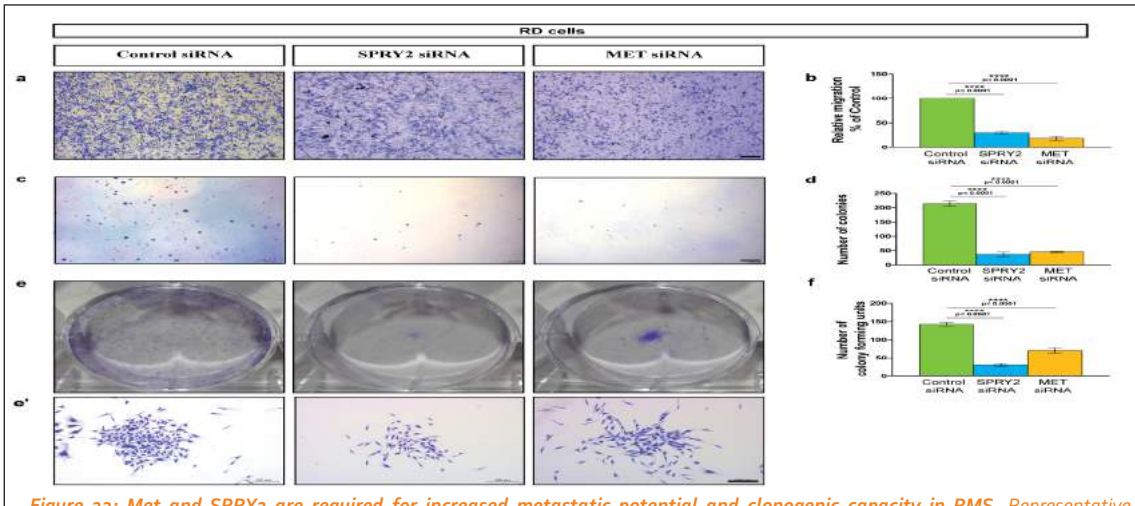


Figure 22: Met and SPRY2 are required for increased metastatic potential and clonogenic capacity in RMS. *Representative images of MET, SPRY2 and control siRNA treated RD cells allowed to migrate for 24 hours in transwell migration assays (**A**) and relative migration was significantly reduced in MET or SPRY2 silenced cells (**B**). Soft agar assay was performed with RD cells post-siRNA treatment to assess the effect of MET or SPRY2 on anchorage-independent colony formation, culturing the cells for 2 weeks (**C**) and the number of colonies formed by MET or SPRY2 silenced RD cells was significantly reduced (**D**). Anchorage-dependent clonogenic assay was performed by culturing MET, SPRY2 or control siRNA transfected RMS cells for 8 days and staining the colonies with crystal violet (**E**, **E'**). Colony formation was inhibited significantly in MET or SPRY2 silenced RD (**F**) cells. The graphical data represent the mean ±SEM. Scale bar in **A**, and **E'** is 100 µm, and is 800 µm in **C**.*

growth (Figure 22C, D) and adherence-dependent colony formation (Figure 22E, E' and F) in ERMS. Thus, our findings indicate that SPRY2 and MET regulate metastatic and clonogenic potential in RMS cells. In addition, we also found that SPRY2 or MET depletion leads to induction of differentiation in RMS cells. The downstream pathway that was most likely mediating the effect of MET and SPRY2 on migratory and differentiation capabilities of RMS was the Extracellular Receptor Kinase (ERK)/Mitogen Activated Protein Kinase (MAPK) pathway. These results thus identify a novel mechanism by which MET signaling is stabilized in RMS by SPRY2 and is a potential target for therapeutic intervention in RMS.

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. We are interested in the MyHCs to understand their expression dynamics and specific roles in skeletal muscle development, differentiation, regeneration and disease. Mutations in one MyHC isoform, MyHC-embryonic, leads to a form of congenital contracture disorder termed Freeman-Sheldon Syndrome (FSS) in humans. We found that the residues most frequently mutated in FSS patients, T178 and R672 are evolutionarily conserved across vertebrates and with the single *Drosophila* MyHC isoform. Therefore, to identify the effect of these mutations on muscle structure, we generated transgenic *Drosophila* overexpressing wild type and mutated MyHC in the *Drosophila* indirect flight muscles. We found that compared to controls, flies expressing wild type or mutated MyHCs exhibited sarcomeric defects such as myofiber branching and defective deposition of Z-disc material.

We are continuing to characterize the function of MyHC-emb during embryonic and neonatal development using a conditional knockout mouse model for MyHC-emb. We 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 future, we will continue our work on RMS to identify the role of specific signalling pathways in regulating RMS cell differentiation and metastasis. We will also extend our in vitro findings to animal models to understand whether MET and SPRY2 play significant tumor metastatic roles in vivo. The MyHC-emb mutations that lead to Freeman-Sheldon syndrome, which are evolutionarily conserved will be studied in detail using the *Drosophila* model, specifically investigating the ATPase activity and functional characteristics of flies expressing the mutated MyHC. Work on the independent functions of developmental MyHCs in skeletal muscle development will continue using in vivo and in vitro approaches. We will also initiate work on the role of MyHC-emb in adult muscle regeneration following injury.

RNA Biology of aging: Towards developing RNA based preventative therapies for late-onset diseases

Dr. Geetanjali Chawla

Principal Investigator

Group member

Konika Choudhury

Aging increases vulnerability to a number of diseases including cancer, diabetes, neurodegenerative and cardiovascular diseases. Restricting nutrient intake without incurring malnutrition or dietary restriction is a robust intervention that can delay aging. This research program aims at assessing the role of small noncoding RNAs termed microRNAs in enhancement of lifespan and reducing risk factors associated with aging. Understanding how evolutionarily conserved RNA mediated networks operate to affect the overall lifespan of an organism will illuminate the basic principles underlying the aging process that can be applied to the development of therapeutic strategies to halt progression of age associated disorders.

MicroRNAs (miRNAs) are a class of small RNAs that negatively regulate gene expression by base pairing to their target mRNAs. A number of human diseases are associated with aberrant expression of microRNAs; and molecules that alter the function or abundance of miRNAs are emerging as potential therapeutic agents to treat diseases. We are interested in understanding the role of non-coding RNA mediated pathways in aging. Use of animal model systems has been inevitable in the study of aging, due to several limitations in using human subjects including length of lifespan, environmental influences, genetic heterogeneity and the fact that researchers themselves are humans.

One such model organism that has yielded valuable insights into the molecular mechanisms of human aging is *Drosophila melanogaster* (fruit fly). Some of the advantages of the use of this model in aging research include shorter lifespan (60-90 days), genetic feasibility, low cost and ease of handling. Thus, this research program is using the fruit fly, *Drosophila melanogaster* as a model to study miRNA mediated post-transcriptional networks that operate during aging and late onset diseases.

MicroRNA mediated mechanisms in aging and dietary restriction

Aging is a major risk factor for a number of non-communicable diseases. Thus, understanding how aging increases the risk of disease, is needed to reduce the prevalence of late-onset pathological conditions (Fig. 23). Towards this goal, increasing evidence has implicated dietary/caloric manipulation – reduced calorie intake that does not incur malnutrition – as a simple means of counteracting age-related disease. Strikingly, this type of nutritional intervention increases lifespan in diverse species, including yeast, nematodes, fruit flies and non-human primates, indicating that the molecular mechanisms that underlie dietary restriction (DR) are evolutionarily conserved. Though this anti-aging manipulation has been

shown to direct profound changes in protein coding RNAs, its effects on noncoding RNA levels remain largely unstudied. Despite growing evidence that miRNAs are altered during aging, there is little evidence on dietary restriction dependent positive effects on miRNAs and their targets or the pathophy-siological consequences of these alterations. This research program is driven by studies that have shown that (i) microRNAs can enhance lifespan and (ii) studies in *C. elegans* and the mouse model that have highlighted the importance of miRNA processing machinery and miRNA regulated networks in mediating dietary restriction-induced longevity. Though these studies have narrowed down miRNAs as key components of dietary restriction pathways, the assessment of the positive effects of miRNAs in promoting organismal metabolic health and their efficacy in functioning as dietary restriction mimetics has not been addressed in *Drosophila* and other model systems. We are addressing this knowledge gap by employing a multidisciplinary approach to identify and characterize the anti-aging effects of conserved miRNAs.

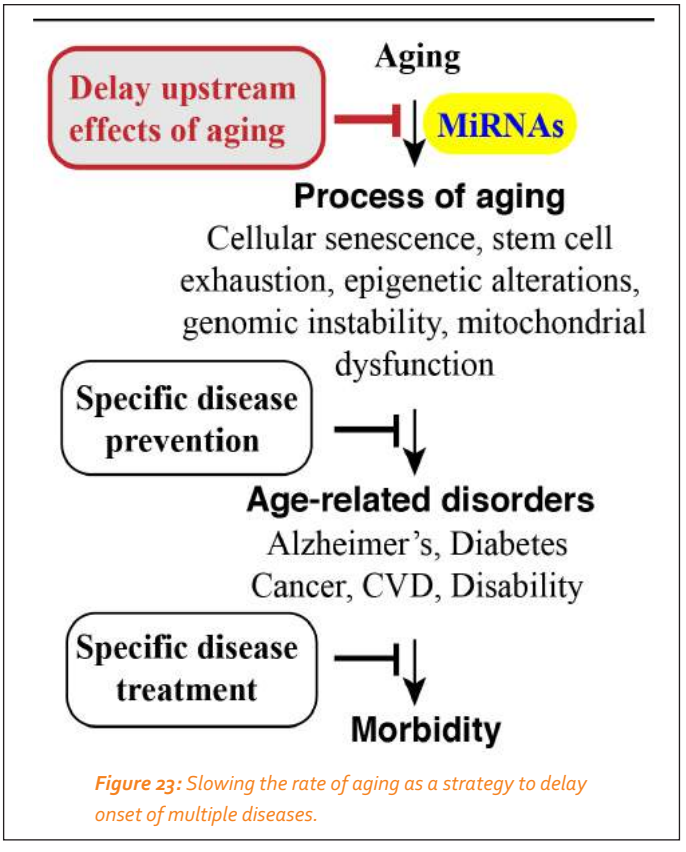


Figure 23: *Slowing the rate of aging as a strategy to delay onset of multiple diseases.*

To assess whether miRNAs can mimic the effects of dietary restriction in animals that are fed a normal diet, we have performed high throughput RNA sequencing of small RNAs isolated from fruit flies exposed to dietary restricted and nutrient rich diets. Since mutations in genes that do not respond to dietary restriction will serve as critical tools for understanding mechanisms underlying lifespan extension, we will be analyzing the lifespan of dietary restriction (DR) and age-modulated miRNA mutants or over expression lines to identify miRNAs that contribute towards DR mediated lifespan extension. Those miRNAs that enhance lifespan will be tested for their ability to lower risk factors associated with aging by utilizing metabolic and stress resistance assays in mutants or overexpression lines. Lastly, we will utilize molecular and proteomic approaches to identify nutrient dependent targets of miRNAs that function as pro-longevity factors. These studies will aid in defining miRNA mediated mechanisms that operate during aging and determine how targeted disruption, competitive inhibition or over expression of miRNA network components modulate aging.

Illuminating microRNA mediated mechanisms that operate during pathogenesis of late-onset neurodegenerative disorders

MicroRNA pathways play a key role in maintenance of post-mitotic neurons. Despite growing evidence that dysfunctional miRNAs lead to neurodegeneration, the assessment of the positive effects of miRNAs in protecting against disease onset has not been addressed extensively in *Drosophila* or any other model system. Previous analysis has shown that loss of activity of two evolutionary conserved miRNAs *let-7* and *miR-125* promotes disease pathogenesis of an age associated neurodegenerative disease model-Fragile X-associated tremor/ataxia syndrome (FXTAS). FXTAS is a late onset human neurodegenerative disease that is characterized by the presence of ubiquitin positive nuclear inclusions containing RNAs with expanded CGG repeats (rCGG) in neurons and astrocytes. The physical symptoms of FXTAS in humans include an intention tremor, ataxia and parkinsonism. De-regulated

expression of transcripts with artificial expansion of these repeats in the fruit fly retina causes photoreceptor degeneration and disorganization of the ommatidia (single units of the compound eye). Using competitive inhibitors termed miRNA "sponges" designed to individually inhibit *miR*-100, *let-7*, or *miR-125* (*miR-100SP*, *let-7SP*, or *miR-125SP*), we tested whether loss of any of these miRNAs' activities enhanced the retinal degeneration in the FXTAS model. Driving *let-7SP* or *miR-125SP* but not *miR-100SP* specifically in the eye resulted in significant enhancement of the rCGG phenotype (Fig. 24). This result indicated that loss of let-7 and miR-125 promotes disease pathogenesis of an age associated disease model. In addition, *Let-7* and *miR-125* expression levels have been shown to be altered in other

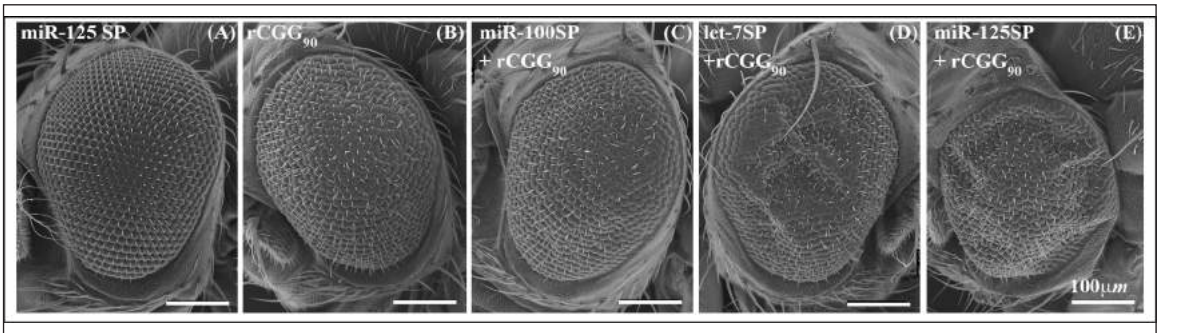


Figure 24: *Loss of miR-125 or let-7 activity enhances rCGG90 mediated retinal degeneration in the FXTAS model. (A-E) Scanning electron microscope (SEM) eye sections from 7d old GMR-Gal4 (eye-specific Gal4 driver) harboring a (A) miR-125 sponge (miR-125SP), (B) a rCGG90 transgene (rCGG90), (C) a rCGG90 transgene along with a miR-100 sponge (miR-100SP + rCGG90), (D) a let-7 sponge (let-7SP + rCGG90) or (E) a miR-125 sponge (miR-125SP + rCGG90).*

disease models as well as in patients suffering from Alzheimer's and Parkinson's disease. However, it is not clear whether these alterations in the expression levels of *let-7* and *miR-125* are etiologically linked to disease, or are a consequence of the disease processes. Thus, continued research efforts are needed to elucidate the beneficial or detrimental effects of specific miRNAs in neurodegenerative pathology and to assess whether sets of miRNAs can be used as a disease modifying treatment of neurodegenerative disorders such as Alzheimer's or Parkinson's disease. We are utilizing molecular, genetic, proteomic and metabolomic approaches to identify and characterize the neuroprotective effects of conserved miRNAs throughout the lifespan of *Drosophila*. Together these studies will focus on characterizing and assessing the contribution of miRNA networks in disease onset and progression of aging neurodegenerative disease models. We intend to translate the findings from the fly projects into mouse models of neurodegeneration through collaborative research, with the long-term goal of developing rational RNA based therapeutic strategies that fine-tune conserved pathways and in addition to providing a broad-spectrum health improvement may also aid in development of a cost-effective way of treating many late onset diseases simultaneously.

Mechanisms of Cell Division and Cellular Dynamics

Dr. Sivaram V. S. Mylavarapu

Principal Investigator

Collaborators

Mahak Sharma, IISER, Mohali
Subba Rao Gangisetty, IISc, Bengaluru
Anjana Saxena, CUNY, New York
Chetana Sachidanandan, CSIR-IGIB, New Delhi
Jayanta Bhattacharya, THSTI-IAVI, Faridabad
Megha Kumar, CSIR-CCMB, Hyderabad

Group members

Kuldeep Verma
Pushpa Kumari
Harsh Kumar
Pergu Rajaiah
Suraj Tewari
Amit Sharma
Amrita Kumari
Sunayana Dagar
Chandan Kumar

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, with malfunctions in these processes leading to deadly diseases. Our group works towards understanding, in molecular detail, how cells carry out the functions of duplication and mutual communication, with the aim of gaining a fundamental understanding of how cells function. Our work is generating a body of knowledge that could be exploited for future therapeutic intervention against major diseases.

Our research group studies the molecular regulation of cellular dynamics. We 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. We aim to understand the dynamic molecular regulation of key cellular processes important in health and disease. As part of this broad aim, we wish to understand the molecular mechanisms of mitotic regulation by the intracellular molecular transport motor cytoplasmic dynein and the mechanisms of membrane traffic during cytokinesis, the final separation of daughter cells at the end of mitosis. Independently, we aim to elucidate the mechanisms of biogenesis and function of novel modes of intercellular communication. We have also begun to also address the mechanistic cell biology of intracellular pathogenic microorganisms. 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 disease conditions.

In the current year, we report major progress made on one of the projects in the lab on the molecular mechanisms regulating cytokinesis, the terminal step of cell division (mitosis), as well as on the elucidation of the cellular biochemistry of a pathogenic virus. The progress made is summarized below.

The Exocyst Complex and Rab5 are Required for Abscission by Localizing CHMP2B to the Cytokinetic Bridge

Cytokinesis, the final step of mitosis ensuring the

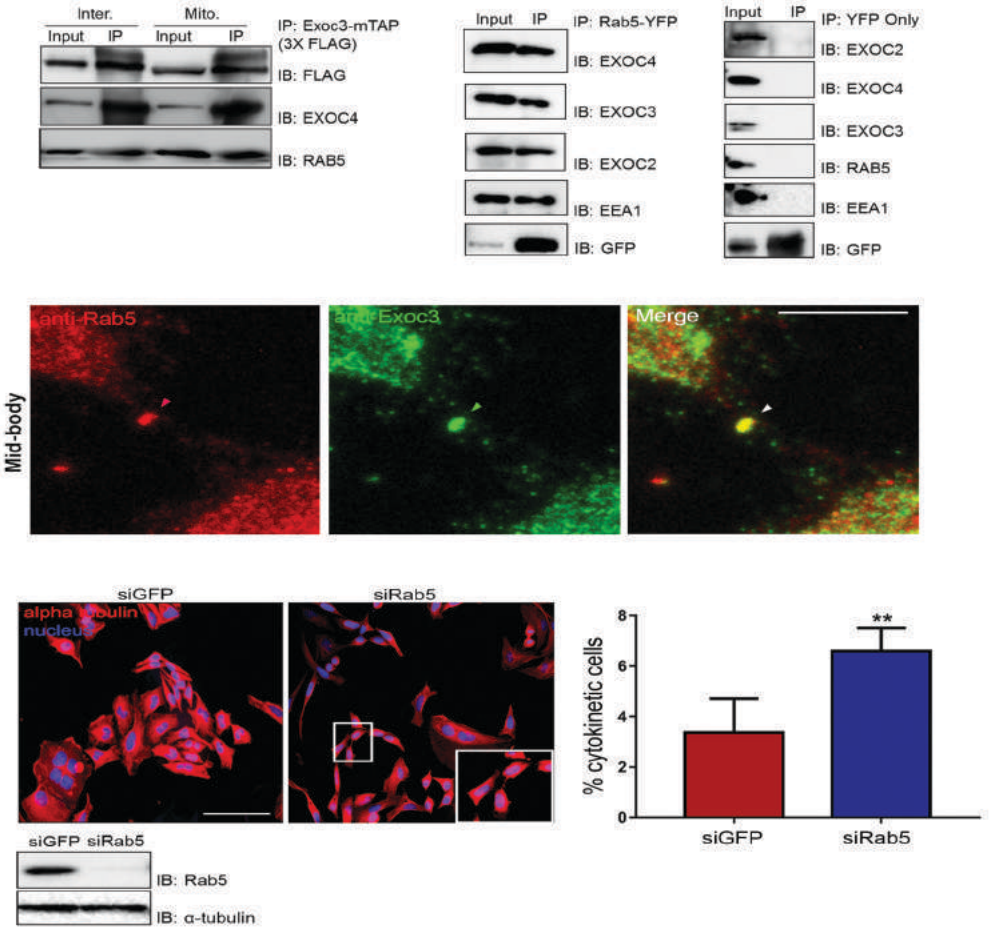


Figure 25: The small GTPase Rab5 is required for cytokinesis. *Top: Representative fluorescence micrographs of Rab5-depleted HeLa cells showing cytokinetic arrest (inset: zoomed image of a cytokinetic cell, arrow points to the midbody region). Red = α-tubulin (microtubules), blue = DAPI (chromosomes). Bottom left: Quantification from three independent experiments for the cytokinetic index (fraction of cytokinetic cells). n=500 cells, error bars represent mean ± SD. Bottom right: immunoblot showing the depletion of Rab5 upon siRNA treatment.*

physical separation of daughter cells, is typified by a sequence of complex subcellular events following karyokinesis (nuclear division) in mammalian cells: cytoplasmic furrow ingression by the cortical actomyosin ring at the spindle mid-zone, formation of a dense proteinaceous structure (midbody ring) in the intercellular bridge, trafficking of membrane vesicles to the midbody region and finally, abscission of the plasma membrane in the bridge leading to separation of the daughter cells. These events are largely conserved across eukaryotes and are controlled by the coordinated action of various proteins. These include centrosome and midbody ring associated proteins (e.g. MKLP-1, Centriolin, BRUCE, Cep55), kinases (*e.g.* Plk-1, Aur B) and regulators of intracellular traffic like Rab GTPases and their effectors, ESCRT complex proteins and the Exocyst complex, the exocytic tethering complex in eukaryotes. The conserved Exocyst complex is required for scission of the cytokinetic bridge connecting the daughter cells, although the molecular mechanisms it employs are unclear.

In this study, we aimed to gain molecular mechanistic insight into the role of the conserved Exocyst complex in cytokinesis. We determined the cellular interactome of the Exoc3 subunit of the complex from U2OS cell lysates. Surprisingly, we found many early endocytic proteins appearing in our eluates, including the small GTPase Rab5. Conversely, affinity purification of Rab5 was also able to pull down

Exocyst complex subunits. Confocal microscopy revealed that Rab5 localized distinctly at the midbody ring in the cytokinetic bridge during late cytokinesis. siRNA-mediated depletion of Rab5 in HeLa cells led to cytokinetic defects akin to Exoc3 depletion as compared to normal cells, confirming a novel function for Rab5 in cytokinesis (Fig. 25). Using live cell fluorescence confocal imaging, we ascertained that the delay was in late cytokinesis at the abscission stage. We set out to determine the precise function of the Exocyst during cytokinesis. One of the ESCRT-III core subunits CHMP2B, a key mediator of cytokinetic membrane abscission, also came up as an interactor of Exoc3, prompting us to explore its link with the Exocyst complex in cytokinesis. Depletion of either the Exocyst subunit Exoc3 or of Rab5 led to the impaired localization of CHMP2B on either side of the midbody ring, suggesting that both molecules were necessary for localization (transport) of the ESCRT-III complex to the cytokinetic site. Depletion of the *Caenorhabditis elegans* orthologs of Exoc3 (SEC6) or Rab5 also resulted in cytokinetic defects in early worm embryos, demonstrating evolutionary conservation of their role in cytokinesis. Overall, the study reveals an evolutionarily conserved role for the early endocytic marker Rab5 in cytokinetic abscission and uncovers a key molecular link between the early endocytic pathway and cytokinesis.

The Exocyst complex maintains steady-state membrane levels of the Notch receptor for normal germline stem cell proliferation

The Exocyst complex is hypothesized to regulate secretory vesicular trafficking in cells by functioning as a vesicle tether at the plasma membrane for post-Golgi vesicles and recycling endosomes. Several components of the secretory pathway are important for viability and fertility in *Caenorhabditis elegans*, and depletion of the Exocyst complex results in embryonic and larval lethality, seamless tube formation in excretory cells, anchor cell invasion during vulval development and dendritic branching. The worm germline undergoes massive growth and morphogenesis as the organism grows to adulthood. Vesicular tethering complexes like the COG were reported to function in the *C. elegans* germline. However, the role of the Exocyst complex in germline development is unknown.

The closed ends of both adult hermaphrodite gonads house the mitotically proliferating germline stem cells (GSCs), which are further enwrapped by the somatic niche cell called the distal tip cell (DTC). GSCs start differentiating through meiosis to form gametes as they exit the niche. Mitotic GSC proliferation depends on the canonical Notch (GLP-1)-Delta (LAG-2) signaling, a conserved signaling pathway required for proliferation in a variety of cell and developmental contexts and in cancers. The Notch receptor expressed on the membrane of the signal receiving cell (here, GSCs) interacts with the membrane anchored Delta-ligand expressed on the juxtaposed signal sending cell (here, the DTC). The Exocyst complex is required for regulating the length of the cellular extensions of the DTC. However, it has not been implicated in regulating Notch trafficking in any system.

We discovered that the Exocyst complex is required for germline development in *C. elegans*. Partial depletion of the *sec-6*, *sec-8, sec-3 or sec-5* Exocyst subunits by RNAi resulted in several germline defects, including a significantly smaller mitotic proliferating zone (MTZ) of GSCs and fewer mature oocytes and defects during oocyte development as characterized by the presence of endo-reduplicating oocytes in the gonad as compared to control worms. Proliferation of GSCs in *C. elegans* is dependent on canonical Notch-Delta signaling. To ascertain whether the Exocyst complex regulated Notch (*glp-1)* signaling in germ cells, we used epistatic analysis of Exocyst with *glp-1* (Notch) mutants, which suggested that the Exocyst complex genetically interacts with Notch to regulate Notch signaling. These and other functional results put together suggested that the Exocyst complex promotes Notch signaling in GSCs.

We wanted to test whether the Exocyst promotes Notch signaling by promoting Notch trafficking in GSCs. We first examined Notch (GLP-1) localization in the germline stem cells in detail. As expected, Notch was localized in discrete punctae along the basal membrane. Surprisingly, we found that Notch was asymmetrically enriched on the cell surface facing the rachis (apical side) at about 1.5 to 2-fold higher levels than at the basal surface in GSCs. We hypothesized that the enrichment of Notch on the apical membranes serves to maintain the steady-state levels of the receptor on the signalling-active basal membrane, thus acting as a reservoir for Notch receptor molecules. We examined whether the Exocyst complex regulates intracellular trafficking of Notch. Upon depletion of the Exocyst, localization of Notch was significantly reduced from the basal membrane and was mostly cytoplasmic. However, the localization on the apical membrane was not affected. The integrity of the plasma membrane, endoplasmic reticulum and the Golgi-body as seen by the respective membrane markers appeared to be normal. A different membrane associated protein SNR-4 (a SNARE protein) also localized normally to the plasma membrane, suggesting that the Exocyst complex is specifically required to traffic Notch to the membrane. These results indicated that the Exocyst complex promotes GSC division by regulating the trafficking of the Notch receptor.

We further investigated the mechanism by which the Exocyst facilitates Notch transport in GSCs. We examined Notch localization in the absence of the early endocytic regulator Rab5. We found Rab5 to be essential for germline development. Interestingly, the GSCs displayed reduced localization of Notch receptor on the basal membrane, as also seen upon Exocyst depletion. The Exocyst is a known effector of Rab11, a regulator of recycling endocytosis. Knock-down of the recycling endosome regulator Rab11 also led to reduction in Notch localization on the basal membrane; however, the apical localization was not affected. Taking together all these results implicate the Exocyst complex in recycling of the Notch

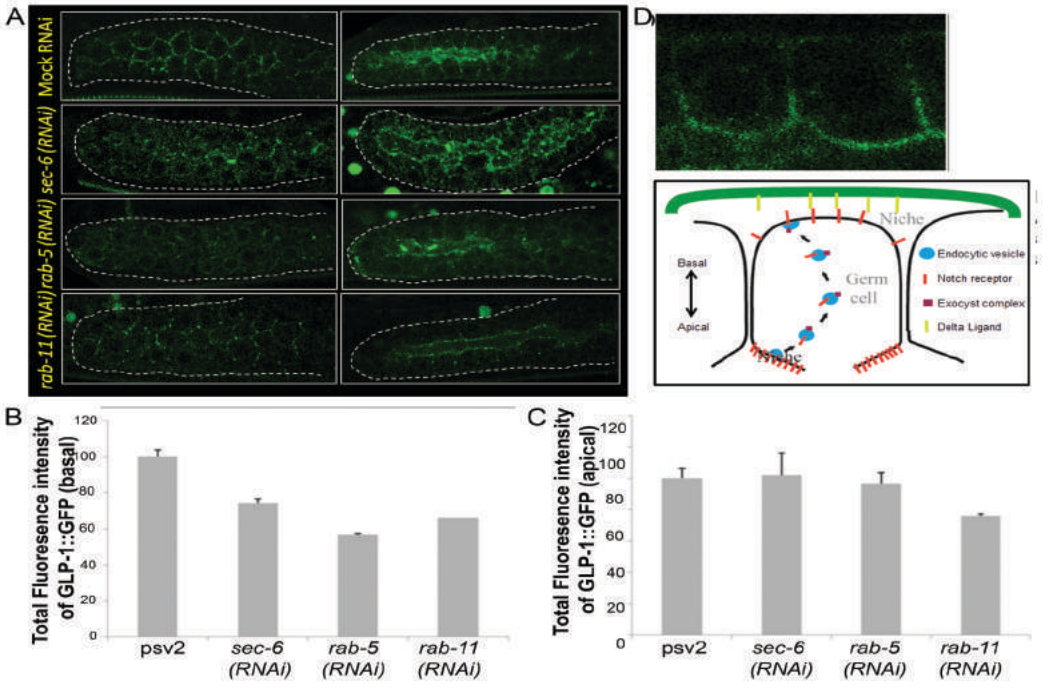


Figure 26: The Exocyst complex regulates trafficking of the Notch receptor. *A. Localization of the Notch receptor in the GSCs of the indicated phenotype. Left panel: apical surface (top plane of the gonad); right panel: Basal surface (middle plane of the gonad). B. Bar diagram displaying the quantification of the total fluorescence intensityof the Notch receptor on the basal surface. C. Bar diagram displaying the ratio of the Notch receptor expressed on the apical vs. basal surface of the GSCs of control and Exocyst-depleted worms. D. Localization of theNotch receptorin GSCs of wild type worms in the middle plane of the gonad (left panel); cartoom representation of Notch trafficking in the GSCs of C. elegans.*

receptor to the basal membrane via Rab5 and Rab11-mediated pathways to enable optimal Notch signalling in the GSCs (Fig. 26).

We hypothesize that a subset of Rab5-positive endosomes recruits the Exocyst complex at a step preceding the maturation of the vesicles to late endosomes. Our immediate efforts are focused on deciphering the molecular mechanisms by which Rab5 recruits the Exocyst complex and to understand how a subset of the endosomal pathway is subverted towards cytokinetic sites by the Exocyst to aid in membrane abscission. We are testing whether the GTPase activity of Rab5 is required for its binding to the Exocyst complex. Our observation that both the Exocyst and Rab5 are required for abscission through their ability to deliver the membrane-constricting ESCRT-III complex at the midbody makes it attractive to hypothesize that the ESCRT complex gets loaded onto a subset of Rab5-positive early endosomes with the help of the Exocyst to facilitate abscission, which we are currently testing.

We have also made key advances towards understanding the cell biology of the pathogenic Chikungunya virus in human host cells as part of a collaborative and multidisciplinary institutional project. Chikungunya virus (CHIKV) is an RNA virus that causes a significant burden during Chikungunya fever epidemics, and is a biomedical problem of both global and national relevance. CHIKV is a positive strand RNA genome virus encoding four non-structural proteins (nsPs) - nsP1, nsP2, nsP3 and nsP4, and five structural proteins - capsid, E3, E2, 6K and E1. The nsPs are important for the genomic replication of viral RNA, while the structural proteins are required for the assembly and integrity of the mature virus. Upon the entry of the mature virus into the host cell, the nsPs along with host cellular proteins replicate its genomic RNA. CHIKVnsP4 is an RNA-dependent RNA polymerase considered a core component of the viral replication machinery that replicates its genomic RNA. However, the entire CHIKV replication machinery and the host cellular proteins that interact with nsP4 are unknown. Using TAP-tagging of nsP4 followed by affinity purification and mass spectrometric analyses, we have successfully identified CHIKV-nsP4 interacting cellular host proteins by mass spectrometry and narrowed down to a small list of nine high confidence and reproducible interactors. This information would be used for understanding the biology of the virus inside host cells and could be exploited for therapeutic intervention.

Engineering of Nanomaterials for Biomedical Applications

Dr. Avinash Bajaj

Principal Investigator

Collaborators

Ujjaini DasGupta, Amity University, Gurgaon

Sagar Sengupta, NII, New Delhi

Group members

Madhurima Mitra
Amit Rajora
Kavita Yadav
Sandeep Kumar
Nihal Medatwal
Sanjay Pal
Animesh Kar
Priyanka Verma
Deepak Mishra
Asad Ansari
Parul Tomar
Rituparna Basak
Ragini Gupta
Sonali Singh
Mohd. Nafees Ansari
Varun Kumar

The Laboratory of Nanotechnology and Chemical Biology (LNCB) is a group of inspired people from chemistry and biology background working together on two major aspects of health issues, cancer and infectious microbial diseases, for the betterment of the health of people suffering from these diseases. We aim at improving the effectiveness of the anticancer drugs and reduce the side effects caused by them either by changing the drug or the drug delivery vehicle. We are also working on making new molecules for identification and treatment of diseases caused by microorganisms like tuberculosis, wound, catheter and urinary tract infections.

We are using interdisciplinary approaches like synthetic chemistry, cell biology, microbiology, cancer biology, nanotechnology, lipidomics, genomics and bioinformatics to address challenges in the area of cancer biology and infectious diseases and to develop nanomaterials for effective therapeutics for cancer and infectious diseases. In this context, here we report (1) Synthesis of gastric pH stable bile acid derived amphiphile where Tamoxifen (as a model anticancer drug) is conjugated to lithocholic acid derived phospholipid (LCA-Tam-PC), (2) *In vitro* anticancer activities, and mechanistic studies of LCA-Tam-PC as compared to parent drug, (3) Antitumor potential, toxicity, and median survival studies of LCA-Tam-PC in murine tumor model, and (4) Pharmacokinetic and bio-distribution comparative studies of LCA-Tam-NBD-PC and parent drug.

Organ toxicity is one of the key parameters that limits the dosage regimens of chemotherapeutic drugs. Therefore, development of new chemotherapeutic modifications with reduced toxicity is much needed for effective cancer therapy. Few chemotherapeutic drugs like Tamoxifen (Tam), a non-steroidal estrogen receptor modulator are used orally in pre- and post-surgical management of breast cancer as an adjuvant therapy. Tam is an aromatic drug with a *N*,*N*′-dimethylaminoethanol side chain; and oral absorption of Tam is hindered by its low dissolution in gastric media. Moreover, poor bioavailability of Tam is exacerbated by its intestinal and hepatic first pass metabolism. Therefore, higher dose administration of Tam is required for effective treatment leading to hepatotoxicity.

Tracing of chemotherapeutic drugs can help in scheduling the proper dosage regimens and can provide advantages in reducing drug-induced

toxicity. Therefore, imaging agents are being integrated with chemotherapeutic drugs for cancer treatment. Oral delivery of imaging probes to monitor the bioavailability of drugs is challenging due to multiple biological barriers of the gastrointestinal tract (GIT) like GIT efflux, acidic pH of stomach, gut microbiota and presence of proteolytic enzymes that can destabilize the delivery vehicles. Therefore, engineering of biomaterials that have the ability to deliver the cytotoxic drugs in combination with imaging agents with enhanced oral bioavailability will be highly useful for scheduling proper chemotherapeutic dosages.

We have synthesized and studied the anticancer activities of a drug-conjugated amphiphile where Tam is tethered to lithocholic acid derived phospholipid (LCA-Tam-PC). We hypothesize that the amphiphilic nature of LCA-Tam-PC would aid in forming mixed micelles in the GIT and assist in better absorption of the conjugate over the parent drug. We also synthesized a phospholipid derived chimeric amphiphile having an imaging probe and Tam conjugated to bile acid phospholipid that would allow easy tracing of the amphiphile to determine the pharmacokinetics and bio-distribution of drugs (Fig. 27).

For synthesis of LCA-Tam-PC, lithocholic acid-tamoxifen conjugate was reacted with 2-chloro-1,3,2-dioxaphospholane-2-oxide followed by reaction with trimethylamine gas in a pressure tube. Purification of the reaction mixture using reverse phase C_{18} silica combi flash column chromatography gave LCA-Tam-PC in 78% yield that was characterized by ^{1}H NMR, ^{31}P NMR, and HRMS. We tested the stability of LCA-Tam-PC in simulated gastric fluid (SGF) conditions for 2 hours and in simulated intestinal fluid (SIF) conditions for 4 hours simulating the normal gastro-intestinal emptying time. HPLC chromatograms of SGF and SIF incubated LCA-Tam-PC revealed that LCA-Tam-PC is stable in both stomach and intestinal media conditions.

Tam, due to its lipophilic character, disrupts the biological membranes apart from its interactions with intracellular receptors. Therefore, it is being used for treatment of early stages estrogen receptor (ER)-positive and ER-negative breast tumors. We tested the anticancer activities of Tam and LCA-Tam-PC against murine (4T1), and human ER-positive (MCF-7) and ER-negative (MDA-MB-231) breast cancer cell lines using the trypan blue cell viability assay. We observed an increase in IC_{50} value of LCA-Tam-PC in all the three cell lines over Tam irrespective of their ER status. The higher IC_{50} of LCA-Tam-PC as compared to Tam might be either due to lower cytotoxicity of the molecule as it is; or slow release of the active Tam from LCA-Tam-PC conjugate. As expected, there was an ~1.5-fold increase in IC_{50} value of Tam and LCA-Tam-PC for triple-negative MDA-MB-231 cells over ER-positive MCF-7 cells due to the absence of ER receptors in triple-negative breast cancer cells. Cell cycle analysis showed a concentration dependent increase in the number of cells in sub G_0 phase of cell cycle confirming the arrest of cells before entering the cell cycle. Apoptosis assays in 4T1 cells using Annexin-FITC/Propidium Iodide (PI) revealed the concentration dependent increase in apoptosis on

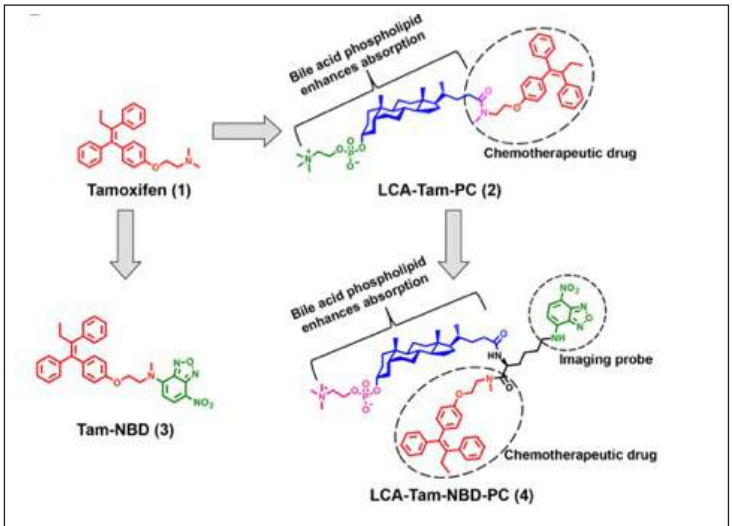


Figure 27: *Molecular structures of Tamoxifen (Tam), LCA-Tam-PC, and their fluorescent derivatives Tam-NBD and LCA-Tam-NBD-PC highlighting the chemotherapeutic drug (Tam) and imaging probe (NBD).*

LCA-Tam-PC treatment as we witnessed an ~9-fold increase in the number of total apoptotic (early and late) 4T1 cells.

We then investigated the relative intracellular uptake of Tam and LCA-Tam-PC by MCF-7 cells using their fluorophore derivatives Tam-NBD and LCA-Tam-NBD-PC. Confocal micrographs revealed a uniform and augmented distribution of LCA-Tam-NBD-PC in MCF-7 cells as compared to poor accumulation of Tam-NBD in the cells. Intracellular mean fluorescence measurements from confocal images confirmed a significant increase in uptake of LCA-Tam-NBD-PC compared to Tam-NBD. We also quantified the cellular uptake of Tam-NBD and LCA-Tam-NBD-PC by flow cytometry after treatment of MCF-7 cells with Tam-NBD and LCA-Tam-NBD-PC at different concentrations. A concentration dependent increase in intracellular levels of LCA-Tam-NBD-PC was observed in comparison with Tam-NBD.

We then evaluated the anticancer activity, toxicity, and survival potency of LCA-Tam-PC suspensions in a 4T1 murine breast cancer model. Tam and LCA-Tam-PC (as per 10 mg/kg equivalent of Tam) were suspended in 0.2 ml (for each mice) of suspending vehicle (0.5% CMC and 0.1% polysorbate-80 in distilled water) for oral delivery. Tumor bearing mice were randomized into four groups of ten animals each. At the palpable stage of tumors; mice groups were administered orally with the vehicle control (suspending agent without drugs), Tam or LCA-Tam-PC suspension at an equivalent Tam dose of 10 mg/kg for three weeks (5 days/week) and one group of mice was left untreated. The tumor volume and body weight of mice were measured on alternate days. We observed an ~60% reduction in tumor volume upon treatment with LCA-Tam-PC as compared to untreated or vehicle control and LCA-Tam-PC was ~2-fold more effective in comparison to Tam (Fig. 28A, 28B). We witnessed an ~2.5-fold decrease in the tumor weight on LCA-Tam-PC treatment as compared to Tam after 21 days of the dosage regimen (Fig. 28C). Survival studies revealed a significant improvement in mice survival where LCA-Tam-PC treated mice showed an ~8 day increase in median survival over control groups (Fig. 28D) without any significant change in body weight (Fig. 28E).

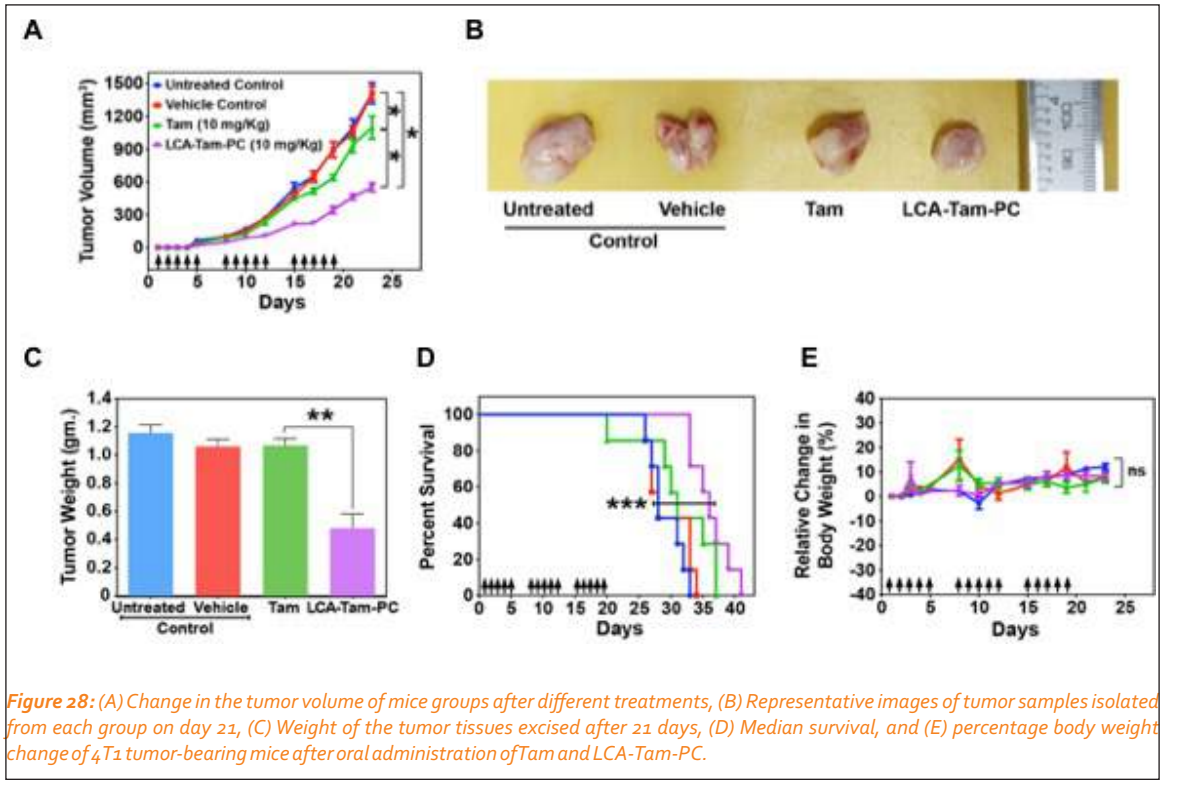


Figure 28: (A) Change in the tumor volume of mice groups after different treatments, (B) Representative images of tumor samples isolated from each group on day 21, (C) Weight of the tumor tissues excised after 21 days, (D) Median survival, and (E) percentage body weight change of 4T1 tumor-bearing mice after oral administration of Tam and LCA-Tam-PC.

We then performed Hematoxylin and Eosin staining of the tumor sections after different treatment regimens to see the effect of these treatments on tumor pathology. Histology analysis of tumor samples in general showed even Hematoxylin staining of nuclei and eosin staining of cytoplasmic proteins. We observed a significant increase in necrotic regions on LCA-Tam-PC treatment with diminished Hematoxylin stain as compared to untreated, vehicle, and Tam treated tumors, suggesting the increased cellular death on LCA-Tam-PC treatment. LCA-Tam-PC treatment induced substantial decrease in the tumor proliferative activity compared to the parent drug as witnessed by a reduced number of Ki-67 positive tumor cells. Chronic oral administration of Tam in breast cancer patients is often linked to hepatotoxicity as evident from an increase in hepatic specific biomarkers like alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP). We therefore estimated and compared the hepatic ALT, AST and ALP levels in mice serum after three weeks of treatment with Tam and LCA-Tam-PC in 4T1 tumor bearing mice. There was a significant increase in circulatory levels of ALT and ALP above the normal levels in mice after Tam treatment. In contrast, we observed normal levels of ALT, AST and ALP in LCA-Tam-PC treated mice. These results suggested that oral administration of LCA-Tam-PC is safe over Tam in chronic treatment schedules.

We then used NBD fluorescent analogues Tam-NBD and LCA-Tam-NBD-PC to estimate the kinetics of Tam in the plasma of 4T1 tumour bearing BALB/c mice. LCA-Tam-NBD-PC demonstrated enhanced $AUC_{0\text{-}24h}$ (840.1 *vs.* 615.5 ng/mL*h) over Tam-NBD (Fig. 29A, 29B). The relative bioavailability of LCA-Tam-NBD-PC was approximately 136.5% in comparison to Tam-NBD confirming the enhanced oral bioavailability of the phospholipid conjugate (Fig. 29C). We then estimated the distribution of Tam-NBD and LCA-Tam-NBD-PC in tumour tissues and other organs after 0.5, 3.0, and 24h of dosing. Fluorescence based quantification revealed an~2-fold increase in NBD concentration at the tumour site post 3h of treatment of LCA-Tam-NBD-PC as compared to Tam-NBD (Fig. 29D). We also observed an ~2.3- and ~1.2-fold increase in NBD concentration in stomach and small intestine respectively as compared to Tam-NBD (Fig. 29D). We then observed the gastrointestinal tissues [stomach, small intestine, colon] for the presence of NBD fluorescence under the confocal microscope after co-staining

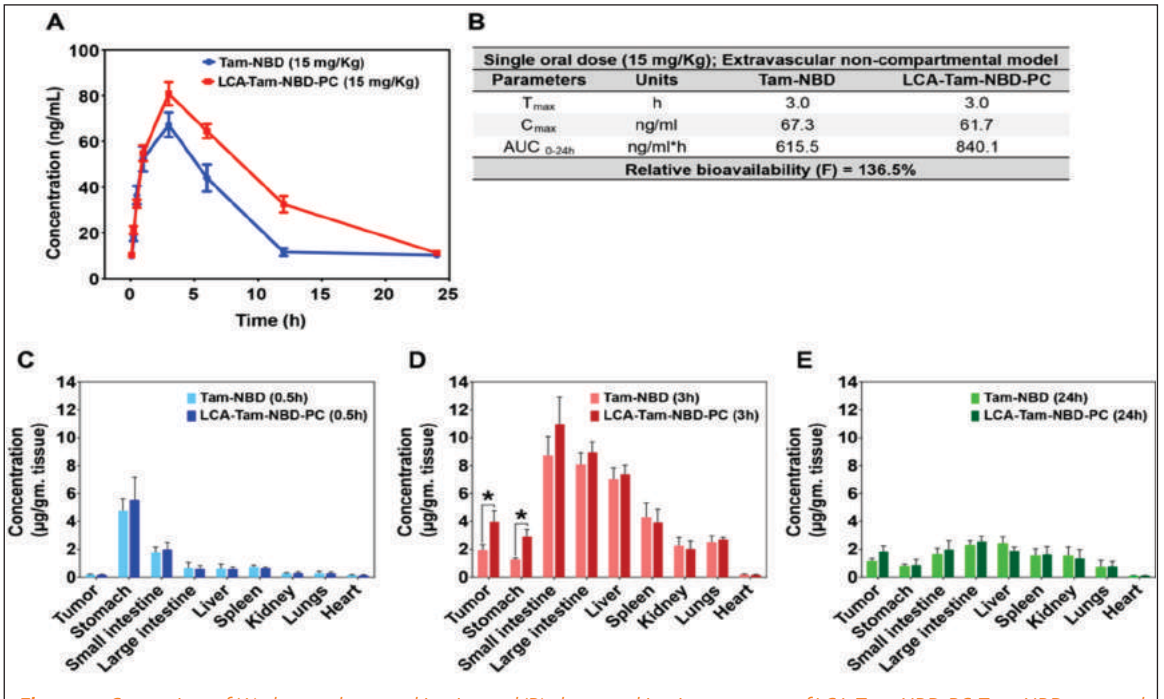


Figure 29: *Comparison of (A) plasma pharmacokinetics, and (B) pharmacokinetic parameters of LCA-Tam-NBD-PC, Tam-NBD upon oral administration of single dose (15 mg/kg, Tam equivalent) of Tam-NBD and LCA-Tam-NBD-PC, (C-E) Comparative distribution of Tam-NBD, and LCA-Tam-NBD-PC in different organs after 0.5, 3 and 24 h of oral administration of single dose of Tam-NBD and LCA-Tam-NBD-PC.*

with Hoechst 33258 (Fig. 29E). Confocal micrographs witnessed the enhanced fluorescence in the stomach and small intestine tissue sections of LCA-Tam-NBD-PC treated mice as compared to Tam-NBD whereas colon sections show a very minimal presence of NBD fluorescence. These results suggested that the maximum amount of LCA-Tam-NBD-PC is absorbed from the stomach and small intestine of GIT as confirmed by bio-distribution studies as well.

In summary, we engineered a lithocholic acid-derived phospholipid-Tamoxifen amphiphile for oral drug delivery and its fluorescent analogue that allowed the easy tracing of the chemotherapeutic drug. The phospholipid-drug conjugate showed enhanced intracellular accumulation as compared to Tamoxifen. *In vivo* anticancer activities established a significant reduction in 4T1 tumour burden in mice on LCA-Tam-PC treatment with reduced hepatotoxicity and increase in median mice survival. Pharmacokinetic and bio-distribution studies using traceable fluorescent analogues confirmed the increased circulatory and tumour-site drug concentrations as compared to parent drug. Therefore, this study gives newer insights into the design repertoire of bile acid phospholipid derived drug conjugates for future cancer therapeutics.

In the future, we plan to engineer phospholipid-derived lipid-drug conjugates using chemotherapeutic drugs for intravenous delivery and to understand the molecular mechanisms responsible for tumour response to a given treatment. In the first plan, we will synthesize phospholipid based bile acid-drug conjugates using different chemotherapeutic drugs. We hypothesize that conjugation of phospholipids to anticancer drugs will help in easy preparation of nanomicelles that can be delivered using the intravenous route. These phospholipid-drug conjugates will then be explored for anticancer activities, pharmacokinetic and bio-distribution studies. We will be undertaking studies to understand the molecular mechanisms underlying the effect of these nanomicelles in different syngeneic tumour models in mice like 4T1 murine breast cancer, CT26 murine colon cancer and murine lewis lung carcinoma models.

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

Dr. Pinky Kain

Principal Investigator

Group members

Sachin Kumar

Srishti Sanghi

Vishakha Chaudhary

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, our lab is interested in understanding 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 *Drosophila melanogaster*, we are 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 modulation of taste behavior. 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.

For all animals, the sense of taste provides the ability to evaluate the quality of food sources and promotes ingestion of nutritious substances and discourages consumption of harmful substances. Our group is interested in the gustatory system of *Drosophila* to understand the feeding behavior, taste circuits and their modulation to achieve the following main objectives: (1) understanding how specific neuronal circuits influence-feeding behaviors, (2) understanding how taste information at the periphery and central nervous system is modulated, and (3) identifying the neuronal pathways that regulate satiety.

Understanding how specific taste neuronal circuits influence feeding behaviors

When the balance between hunger and satiety is perturbed, food intake gets misregulated, leading to excessive or insufficient eating. In humans, abnormal nutrient consumption causes metabolic conditions like obesity (causing 3

million deaths/year) and eating disorders. Despite this burden on society, we currently lack enough knowledge about the neuronal pathways, circuits and genes that regulate appetite.

By exploiting the gustatory system of the flies, the lab is interested 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. By using experimental strategies involving molecular, genetic, calcium imaging and electrophysiological approaches we are interested in dissecting the taste neural circuits (especially higher order taste neurons) that convey taste information to the brain and are involved in simple feeding behaviors like acceptance or rejection of food (Fig. 30A). To address this, we have screened around 150 promoter GAL4 lines by expressing GFP to identify taste circuits only in the brain (Fig. 30B). Expression of temperature sensitive cation channel dTrpA1 to activate newly identified neurons with different GAL4s caused >50% proboscis extension response (first step in appetitive behavior) at 32° C in 21 GAL4 lines marking different sets of neurons that might be involved in the appetitive behavior and are potential candidates for sugar sensing (Fig. 30C). We are now in the process of delineating these unmapped taste neural circuits in the brain with high cellular resolution and calcium imaging approaches to monitor taste-induced activity and understanding their physiological roles. 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.

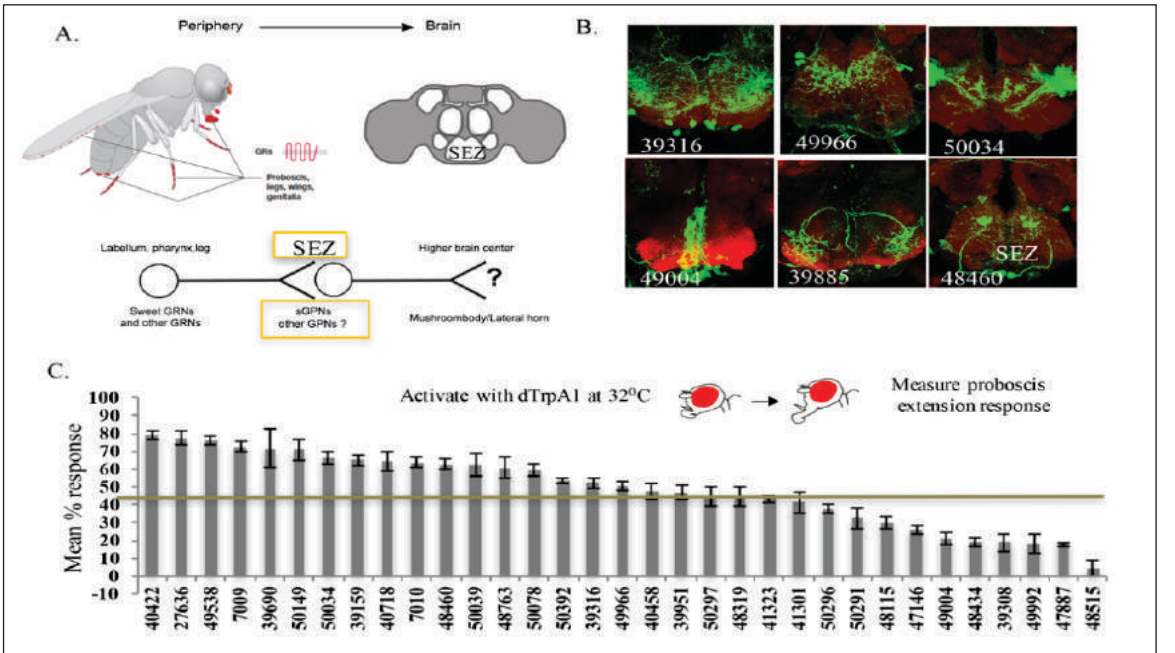


Figure 30: *(A) Drosophila peripheral and central taste organs. Peripheral taste organs of adult Drosophila express various taste receptors in taste neurons of different taste organs (labellum, legs, wings and genitalia) and send projections of Gustatory receptor neurons (GRNs) to brain in the sub esophageal zone (SEZ). SEZ is a first relay for taste information. The identity of second order gustatory projection neurons (GPNs) that respond to different taste modalities is still unknown except one sweet and one bitter projection neuron (Kain and Dahanukar, Neuron, 2015, Bohra et al., Current Biology, 2018). (B) Identification of neuronal pathways involved in the feeding behaviors. Expression pattern of some of the newly identified neurons only 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. (C) Proboscis extension response (PER) of flies at 32 °C by expressing Drosophila heat activated channel- dTrpA1 with various GAL4 lines. 21 GAL4 lines showed PER response around 50% and above that could be involved in the appetitive behavior. Otherwise for all genotypes, we observed little proboscis extension at 22 °C.*

Starvation state and neuronal activity in sensitized pharyngeal neurons induce modulation of salt taste behavior in *Drosophila*

Sodium present in the salt (NaCl) is a fundamental nutrient that is required for many physiological processes, the most important ones include electrolyte homeostasis and neuronal activity in the body. The worldwide salt consumption is approximately 8.0 g of salt per day, far more than what we need, putting us at risk of various health problems like blood pressure, strokes, bone weakening, stomach cancer, osteoporosis, obesity, kidney stones, vascular dementia and water retention.

Little evidence exists for a genetic determination of individual differences in consumption and preferred level of salt. 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. To understand how early experience with high salt diets may have a long-term impact on preferred salt levels, other taste preferences and modulation of taste behavior, we are looking into the molecular mechanisms of high salt feeding in *Drosophila* which has not been explored so far.

In animals including mammals and *Drosophila*, the detection of NaCl produces two different behaviors in a concentration dependent manner: low-salt concentrations (<100 mM) act as an attractive cue and induce attractive behavior, whereas high-salt concentrations (>200 mM) evoke aversive behavior. In our study, we found that flies on high NaCl (200 mM) diet show developmental delay, lay less eggs and die faster as compared to flies feeding on normal media (Fig. 31A-D). Our two-choice feeding and proboscis extension reflex assay (Fig. 31E-2J) results suggest that flies are attracted to low levels of salt (50 mM) and eat less salt when presented in higher concentrations (>100 mM).

We have identified that flies pre-exposed

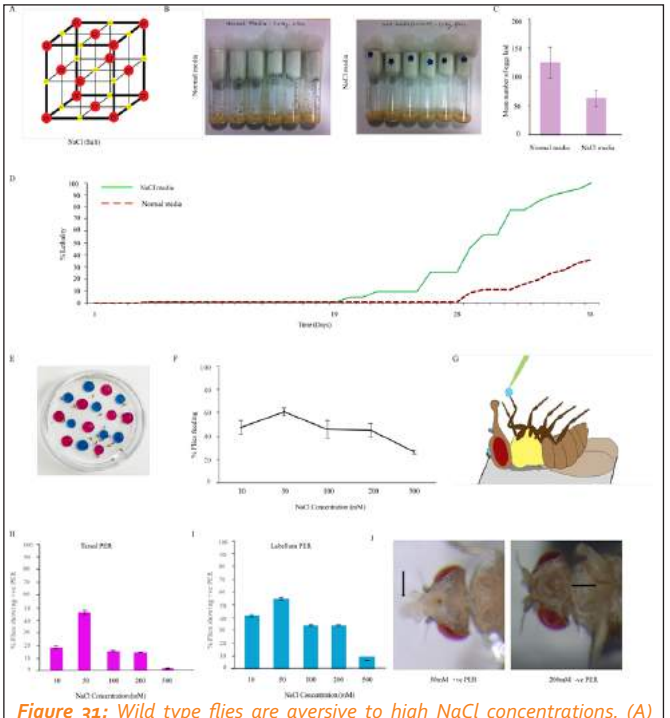


Figure 31: *Wild type flies are aversive to high NaCl concentrations. (A) Structure of NaCl (Table salt). (B) Wild type flies (CsBz) growing on high salt media (200 mM mixed in the fly media) show developmental delay. Flies eating high salt throughout the development take more time to eclose at 25oC (14-15 days) and grow slower than the control flies on normal media (10-11 days) (N=6 vials in both conditions). (C) Flies on high salt lay less number of eggs than control flies on normal media (N=6 plates in each case). (D) Wild type flies feeding on high salt have shorter life span. Freshly eclose wild type flies are transferred on high salt mixed with normal media and in case of control on regular fly media (flies transferred regularly every 3rd day on fresh media conditions during the assay). Percent lethality is calculated for 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) when compared to control normal media flies (60-90 days). (E) Two choice feeding assay for adult flies. In our two-hour assay, flies are allowed to make a choice between water and varying concentrations of salt in an unbiased manner in the dark. Blue is just water and agar. Red is salt added with ager and water. % flies eating salt is scored based on abdomen color. (F) Dose response curve for salt (N=6 plates for each concentration, 20 flies- 10 males and 10 females each plate). Wild type flies (CsBz) showing highest attraction towards salt at 50 mM concentration and aversion at high salt concentrations. (G) Proboscis extension reflex assay showing flies extend their proboscis in response to sugar drop presented to the tarsal hair (Tarsal PER) or to the labellum taste hairs (Labellum PER). We mount fly facing upwards on a glass slide with the help of a nail polish. (H and I) Both tarsal and labellum PER show similar trend of responses (N= 60 flies, 20 X 3 slides for each concentration). Highest PER responses are observed at 50 mM salt concentration as in case of plate assays. During this assay flies are not allowed to drink the solution. (J) Real time snapshot of tarsal PER. Black arrow showing proboscis extension at lower salt concentration and non-extension at higher concentration.*

to high salt diet for three days (200 mM NaCl mixed with normal fly media) get sensitized and maintain their preference for high salt (100, 200 and 500 mM NaCl) even afterwards (Fig. 32A). Such sensitized flies prefer selective sugars that can be easily metabolized to fulfill their nutrient and energy requirements (Fig. 32B). Our results suggest that mated females have a higher appetite for salty food and their weight increase after feeding on salt diets (10 to 200 mM concentrations) as compared to mated males (Fig. 32C-D). Our spectrophotometry analysis revealed that mated females consume more water after feeding on salty diets than mated males (Fig. 32E). Our results suggest that mated females have a high demand of sodium ions in their diets and eat more salt for laying eggs and to supplement the progeny with sodium ions.

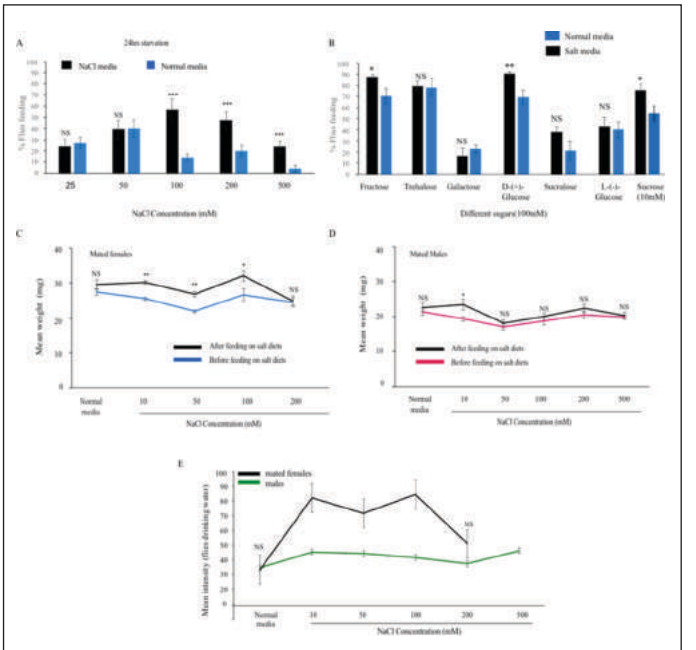


Figure 32: *(A) Flies pre-exposed to high salt (200 mM in the fly media) maintained their preference for high salt concentrations including 100, 200 and 500 mM NaCl even later (black bars) as compared to normal media fed flies (blue bars). No changes in feeding were observed at low concentrations 10 and 50 mM NaCl between normal and salt media fed flies. For these feeding plate assays flies are starved for 24 hrs. (B) flies on high salt diet choose to feed on selective sugars which metabolize faster like fructose, D glucose and sucrose (10 mM) but not trehalose, Galactose, non- nutritive sugar Sucralose and L-(-) Glucose when tested at 100 mM concentrations in each case. (C and D) After eclosion followed by mating for 3 days, 4 days old flies were separated as males and females, and were grown on different concentrations of salt mixed with normal media for the next 3 days. Weight of the flies was taken before and after consuming salt media. The increase in mean weight of the females at concentrations 10, 50 and 100 mM NaCl suggest that mated females feed more on salt diets than mated males. We did not see any significant difference at 200 mM NaCl. Female flies died on 500 mM NaCl concentration while feeding for 3 days and could not be used for weight analysis. In case of males only small difference in mean weight was observed at lowest concentration 10 mM (D). The weight of the male flies at 50, 100, 200 and 500 mM found more or less similar before and after feeding on various concentrations of salt in their diet. (E) Spectrophotometry analysis suggest female flies (Black bars) consume more water than males (Green bars) after feeding on salt diets. In this assay, after feeding flies were desiccated for 5 hours, then these flies were left in the vials with wet tissue papers for 2 hours so that they can consume water (mixed with blue dye). Flies feeding on normal media were used as a control set. For doing the spectrophotometry analysis, 50 flies in each case (n = 50 in each set, sets-3) were tested to take the reading at 630 nm. Mean intensity is calculated for each case after taking the readings at 630 nm 5 times for the same set.*

We have found that *Drosophila* ionotropic receptor Ir76b (already shown to be required for attraction to low salt- NaCl food, *Zhang et al., Science, 2013,*) and neurons (silencing neuronal activity by expressing active form of tetanus toxin- TNT and by using *Ir76b* mutants- *Ir76b* ^{51310} *and Ir76b* ^{51309}) are both responsible for detecting high concentration of salt but not for maintaining preferences towards high salt feeding in flies pre-exposed to high salt under starvation conditions. Our data suggests that Ir76b acts as a channel for detecting both low and high salt levels. Mechanisms that regulate high salt intake are different from the Ir76b pathway.

Our study also suggests the novel role of sweet pharyngeal neurons of LSO (Labral sense organ) in regulating the high salt intake. Pre-exposure to high salt diet followed by 24 hrs starvation leads to sensitization of normal active sweet sensing pharyngeal neurons in the absence of activity in peripheral sweet neurons present on the labellum (Fig. 33A, *Gr5a-GAL4>UAS-TNT*). This causes higher feeding on salt in sensitized flies both at 50 mM and 200 mM concentrations. Silencing the activity of sweet neurons at the periphery and in the pharyngeal LSO neurons by UAS-TNTG driven by Gr64a, Gr64e, Gr64f, Gr43a GAL4 lines leads to normal salt feeding and does not cause sensitization like control flies suggesting active sweet pharyngeal neurons

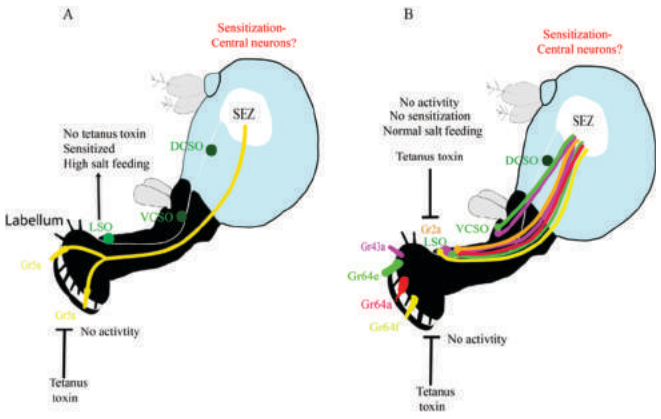


Figure 33: *Three distinct internal taste organs are present in the adult fly pharynx: the labral sense organ (LSO), the ventral cibarial sense organ (VCSO), and dorsal cibarial sense organ (DCSO). In wildtype flies high NaCl feeding followed by starvation causes sensitization and higher feeding of salt and selective sugars. (A) Absence of activity at the periphery by tetanus toxin (Gr5a neurons > TNT) results in high salt feeding in starved flies. Active sensitized pharyngeal sweet neurons of LSO lead to higher salt feeding. Sweet taste dominates in LSO area (Chen and Dahanukar, Cell Reports, 2017) suggesting unsensitized normal active sweet sensing pharyngeal neurons in LSO maintains and regulates high salt intake. (B) The high salt feeding and sensitization get suppressed in the absence of activity at the periphery and in the pharyngeal sweet neurons (Gr43a, Gr65a, Gr64e, Gr64f and Gr2a express in labellum and LSO). The role of central neurons that get sensitized by salt feeding and in general identity of higher order neuron for salt is an open area to explore. Our results suggest that reciprocal regulatory mechanisms on salt taste sensitivity may exist in pharyngeal taste organs like LSO, contribute to salt intake and play an important role in sodium homeostasis.*

regulate the high salt intake in flies. Similar results were observed when salt sensing Gr2a (which expresses only in LSO neurons) pharyngeal neurons were silenced by tetanus toxin (Fig. 33).

Our result suggests that pre-exposure to salt enhances sweet taste sensitivity for only selective sugars and for low as well as high salt. Our results also present different mechanisms where the Ir76b channel (both receptor and neurons) is involved in the detection of high NaCl, low and high levels of sodium benzoate. Unsensitized normal active sweet sensing pharyngeal neurons maintain and regulate the salt intake. Sensitized pharyngeal sweet neurons lead to higher salt feeding. The high salt feeding and sensitization gets suppressed in the absence of sweet pharyngeal neuronal activity (Fig. 33). Our results suggest that reciprocal regulatory mechanisms on salt taste sensitivity may exist in pharyngeal taste organs like LSO, may contribute to salt intake and play an important role in sodium homeostasis. Furthermore, the linkage between salty and sweet taste modulations may optimize sodium and calorie intakes. Understanding the mechanism of high salt feeding and its effect on feeding behavior, longevity, mating and egg laying behavior in insects like *Drosophila* could help in preparing inexpensive and effective pesticidal salt baits for pest control.

Understanding age related changes in our 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 the age of 60 in humans. In addition, our mouth produces less saliva as we age. This can cause dry mouth, which can affect our sense of taste. Decreased taste and smell can lead to less interest, diminished appetite and no enjoyment while eating. In our preliminary two choice feeding assays, we have found less sugar feeding 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. Using *Drosophila*, we are now trying to understand the common markers of aging and diseased conditions that induce modulations of taste behavior. Understanding the age-related factors affecting taste can help us prepare to accept change, adapt, and be aware of potential hazards and help in aging gracefully with changed heathy eating habits.

In the future, we are interested in investigating the role of different physiological states like hunger and thirst that might alter the responses of newly identified candidate neurons and various gustatory receptors. The feeding responses of flies will be assessed under food and water deprivation conditions.

We will be comparing our results 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 lab is interested in exploring how hunger and satiety are achieved by identifying pathways, neurons and genes involved and relate our findings to homologous mammalian genes with similar functions to discover conserved pathways that regulate hunger and satiety.

Exploration of molecular intricacies that regulate and execute effector-triggered innate immunity in plants

Dr. Saikat Bhattacharjee

Principal Investigator

Group members

Yashika Walia Dhir
Jewel Jameeta Noor
Hitika Gulabani
Ingole Kishor Dnyaneshwar
Krishnendu Goswami
Mritunjay Kasera
Shraddha Dahale
Abhisha Roy

Plants have evolved a sophisticated defense system to combat the threats posed by the constant risk of pathogen attacks. This intricate immune network cleverly balances the energy requirements of a defense response through proportional adjustments of regular growth and developmental functions. At the molecular level, proteins that serve the dual function of associating with not only defense mediators but also coordinating via signaling messengers with cellular homeostasis processes are predominantly recruited. We explore characterization of these signaling routes with a broad aim to improve biotechnological approaches aimed at generating disease resistant crops.

Defense responses in plants involve intricate signal transduction networks that impose adjustments on processes that promote regular growth and development. We trace molecular mechanisms of immune triggers, signaling routes and its execution with a broad aim to biotechnologically improve 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 response 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. We investigate the role of inositol phosphates (InsPs) and lipid-conjugated InsPs - the phosphatidylinositols (PtdIns) as signaling mediators of plant defenses. Our research is focused on (1) Characterizing the strategic deployment of immune complexes on PtdIns-related interfaces and their mode of modulation by pathogenic effectors, (2) Deciphering defense signaling routes mediated by InsPs, and (3) Elucidating defense response-imposed impingement on regular developmental processes and their crosstalk.

Plant responses to pathogens are highlighted by massive restructuring of its transciptome. While it is anticipated that the majority of expression changes are aimed towards thwarting the

pathogen, gene ontology classification of transcripts undergoing expression alterations does not entirely bias on defense-associated genes. Instead, a global reprogramming, which often includes networks that regulate physiological processes such as photosynthesis, cell division, phytohormone signaling and general growth and development is equally represented in expression changes. Extensive studies during the last several decades provide a strong understanding to these responses. Undoubtedly, preventing invasion is a clear aim of the plant but parallel adjustments that can adapt to the energy demands of defense are conditioned to maintain minimal fitness cost to the host. However, a multifaceted signaling messenger(s) that orchestrates this remains elusive. Ever since the identification of inositol phosphates (InsPs), their versatility in cellular signaling is ever-increasing. The six-membered cyclic carbon ring (myo-inositol) with its six hydroxyl groups allows variable number of phosphorylation (and pyrophosphorylation) events thereby generating different InsPs. These diverse InsPs, depending on the degree and position of phosphorylation, can function as agonist or antagonist co-factors thus modulating responses based on their relative ratios. In plant defenses, the involvement of InsPs remains unexplored. We are undertaking detailed investigations into the roles of specific InsPs in defense responses. We explore the model plant system *Arabidopsis thaliana* and the devastating hemi-biotroph *Pseudomonas syringae pv* tomato (strain DC3000) pathosystem for our investigations. Individual approaches undertaken and their progress during the past year are as follows.

Higher InsPs are mainly involved in the regulation of defense responses

We had earlier tested the pathogenicity of the virulent *P. syringae* strain on defense elicitations in several InsP-biosynthesis and metabolism mutants (Fig. 34). However, at least three mutants remained untested due to a lack in the availability of appropriate seeds. These include knockout in *ITPK2* (an isoform of ITPK-type InsP kinase), *VIH1* (responsible for synthesis of $InsP_7$) and *VIH2* (responsible for synthesis of $InsP_8$). We obtained seeds of *vih2-4* and *itpk2-1* from our collaborator Prof. Gabriel Schaaf, Univ. of Bonn, Germany. Remarkably in pathogen growth curve assays on *vih2-4* plants, we observed elevated levels of basal resistance to virulent *P. syringae* (Fig. 34). This, we conclude is a supporting evidence of antagonism between jasmonic acid (JA) and salicylic acid (SA) defense networks. Indeed, deficiencies in JA-pathways have been reported earlier for *vih2-4* plants. However, taking into account a previous report that suggested that JA-pathways are enhanced in *ipk1-1* plants, we find enhanced resistance in this mutant intriguing. We are performing defense network elucidations to determine underlying causes of increased resistance in *ipk1-1* and *vih2-4* and how they cause differences in JA-responsive pathways.

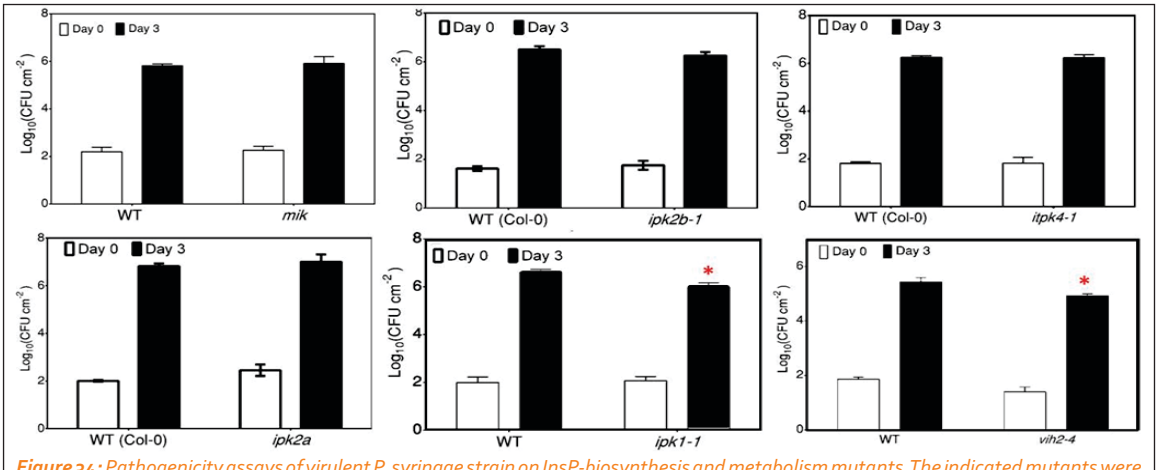


Figure 34: *Pathogenicity assays of virulent P. syringae strain on InsP-biosynthesis and metabolism mutants. The indicated mutants were syringe infiltrated with the bacterial resuspension and growth of bacteria estimated on Day 0 and Day 3. In each case, the comparison was determined to bacterial growth on wild-type (Col-0) plants. Asterisk indicates statistically significant reduction in bacterial growth in ipk1-1 and vih2-4 plants.*

We further profiled InsP changes (with help from Dr. Schaaf) in various InsP-biosynthesis mutants to identify specific InsPs regulating immunity. These profiling assays show that basal $InsP_6$ levels are reduced in *mik1*, *ipk2β-1* and *itpk4-1* although $InsP_7$ levels are comparable to wild-type plants (Fig. 35). Thus, the categorization of these mutants as *bona fide* low-phytic acid (*lpa*) mutants is valid; however, pathogen susceptibilities of these mutants remained comparable to wild-type plants. The *ipk2α* mutant has been reported earlier to contain wild-type levels of different InsPs. Surprisingly *ipk1-1* which also has low $InsP_6$ in sharp contrast displays enhanced resistance to *P. syringae* suggesting that defenses in these plants are $InsP_6$-independent. We are investigating whether higher InsPs, $InsP_7$ and/or $InsP_8$, which are reduced in *ipk1-1* plants, define its pathogenesis outcome. Nevertheless, it is clear from our assays that both VIH2 and IPK1 function as negative regulators of innate immunity in *A. thaliana*.

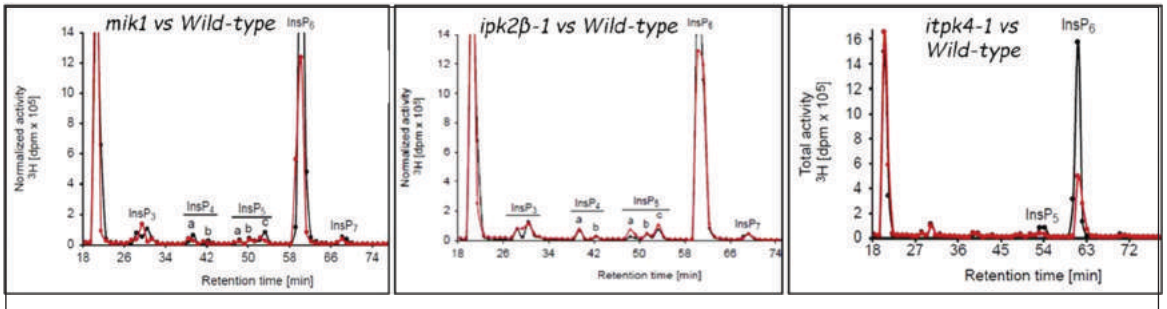


Figure 35: *mik1, ipk2β-1 and itpk4-1 are bona fide low-phytic acid (lpa) mutants. Extracts from [3H] inositol-labeled plants were analyzed by SAX-HPLC for relative levels of different InsPs. Red and black line profiles in each panel is for the corresponding mutant and wild-type plants, respectively. The measurements were done as a collaborative effort with laboratory of Prof. Gabriel Schaaf, U. Bonn, Germany.*

The pathogen effector $HopA1_{pst}$ evades plant defenses likely by targeting distinct lipids

Initiation of defense signaling requires a pathogen-originated trigger. These include pathogen-associated molecular patterns (PAMPs) and effectors secreted into the plant cytoplasm. We are characterizing the virulence function of one such class of effectors, HopA1. Two pathovar-specific HopA1 are perceived differently in *A. thaliana* ecotype Columbia (Col-0). HopA1 from pv *syringae* ($HopA1_{pss}$) elicits ETI whereas HopA1 from pv tomato ($HopA1_{pst}$) does not. Several reports suggest that rapidly evolving HopA1 in *Pseudomonas* pathovars is one of the main causes of expansion of its host range and increased infectivity on economically important crops. In continuation with our previous demonstration, we have identified that the two HopA1s bind overlapping yet distinct classes of lipids *in vitro*. While phosphatidic acid (PA) is bound by both HopA1s, phosphatidylserine (PS) is only bound by $HopA1_{pst}$. Lipid compositions in a cell are tightly regulated and cued changes are responsible for intracellular communications. One such mode of signaling is achieved through lipid droplets (LDs). Animal pathogens such as Hepatitis C, Dengue virus, and *Chlamydia* infections increase LD accumulation either as an anti-defense strategy or to obtain nutritional resources from the host. Because PA and PS modulate LD-biogenesis, we are testing whether the two HopA1s utilize this mode in their virulence functions. $HopA1_{pss}$ induces stronger defenses than $HopA1_{pst}$ in *Nicotiana benthamiana*. Upon transient expression followed by staining with the lipophilic neutral lipid-staining dye BODIPY (4,4-difluoro-3a,4adiaza-s-indacene), we detected increased LD accumulation in both HopA1 infiltrations than the mock control (Fig. 36). Curiously, $HopA1_{pst}$ elicits significantly more LD formation than $HopA1_{pss}$. Considering inverse correlation of LD numbers to defense responses, it is suggestive that $HopA1_{pst}$ is more competent in suppression of immune responses in *N. benthamiana*. We are pursuing domain mapping of these two HopA1s that cause distinct lipid-binding and hence the difference in immune responses. We envision that our approach would provide deeper insights into defense-evading evolution of $HopA1_{pst}$.

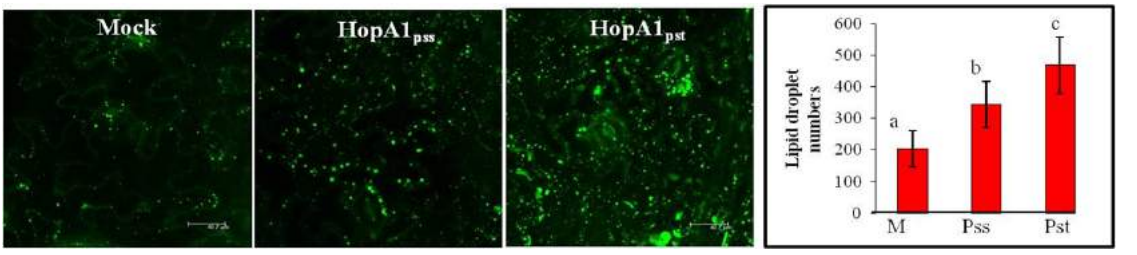
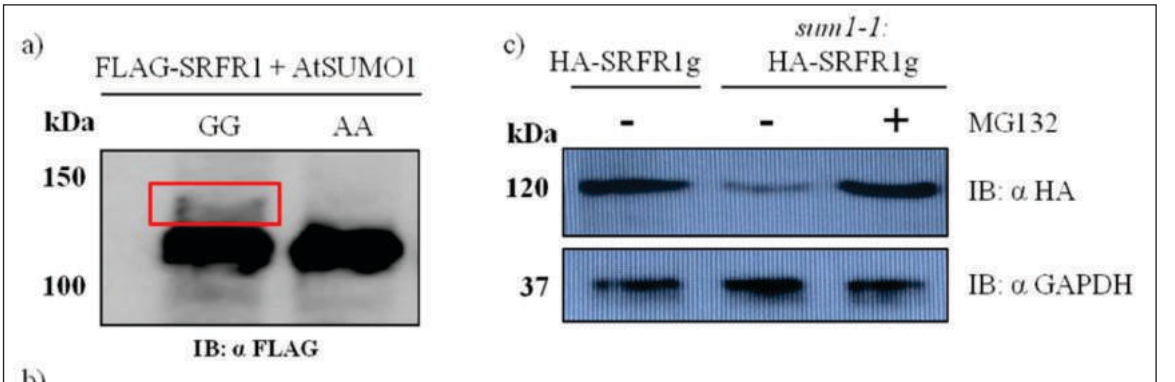


Figure 36: Transient expression of HopA1 increases lipid droplet accumulation in Nicotiana benthamiana. Epitope tagged HopA1pss and HopA1pst were expressed in leaves via Agrobacterium-mediated infiltration. As a control leaves were also infiltrated with buffer alone. At 3-dpi leaves were stained with lipid dye BODIPY and imaged under a confocal microscope (left three panels). Quantitation of lipid droplets numbers were done via microscopy imaging software Imaris (right panel). Lipid droplet numbers in each type of infiltration were statistically different from the others (P <0.05). M: Mock; Pss: HopA1pss; Pst: HopA1pst.

AtSUMO1 SUMOylates and regulates the stability of SRFR1

Most immune modulators undergo extensive post-translational modifications (PTMs). This ensures their deployment to multiple cellular locales and facilitates interaction with diverse sets of proteins that eventually lead to efficient sensing of pathogen effectors to mount appropriate defenses. The identification of resistasome complex provided strong support to its strategic deployment and also provided mechanistic insights into perception of pathogen effectors. However, the assembly process of this multi-component complex is not known. We previously demonstrated that SRFR1 is a target of PTM by SUMOylation. Interestingly, three *A. thaliana* SUMO isoforms AtSUMO1, AtSUMO2 and AtSUMO3 were shown to *in vitro* SUMOylate SRFR1. Although bio-informatic analysis predicts multiple SUMOylation motifs in SRFR1, actual demonstration of covalently modified lysine (K) residues are necessary. Towards this end, we enriched SUMOylated SRFR1 and have performed mass-spectrometric analysis to identify SUMOylated K residues. We identify K^{229} and K^{325} as two potential Ks



b)

Protein	Peptide detected	Modification
SRFR1 @K229	CGSQINGYYESCKPCNGSDLHDNLAESSDR	GlyGly(K)@13
SRFR1 @K325	VLKEEPTYPEALIGR	GlyGly(K)@3

Fig. 37: Selected SRFR1 lysine residues are SUMOylated by AtSUMO1 in vitro. SRFR1 is targeted for proteasome-mediated degradation in sum1-1 plants. FLAG-tagged SRFR1 was expressed in E. coli SUMOylation system in the presence of SUMOylation-proficient AtSUMO1 (GG) or SUMOylation-deficient AtSUMO1 (AA) (panel a). Immunoblot was performed with anti-FLAG antibodies. Red-boxed fragment (panel a) was subjected to MS analysis and AtSUMO1 footprints on SRFR1 detected (Red K; panel b). HA-SRFR1 expression was compared in wild-type and sum1-1 background by immunoblot with anti-HA antibodies (panel c). Further on extracts from sum1-1: HA-SRFR1g plants were prepared in the absence and presence of proteasome inhibitor MG132. Immunoblots for equal extract loading was performed with anti-GAPDH antibodies.

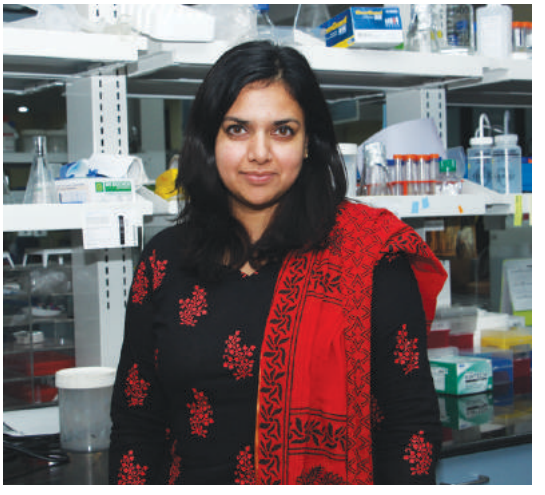
which undergo covalent modifications by AtSUMO1 (Fig. 37a). The LC-MS/MS analysis clearly detects di-glycine footprints of AtSUMO1 on these residues (Fig. 37b).

SUMOylated targets undergo different fates as a consequence of this modification. Considering that SRFR1 as a candidate of SUMOylation, its fate and hence its subsequent impact on the immune state of the plant posits an important avenue to investigate. Towards this, we have generated an epitope-tagged, native promoter-driven SRFR1 genomic clone (*HA-SRFR1g*) via crossing into the *sum1-1* mutant. A *sum1-1* plant contains a knock-out mutation in the *AtSUM1* gene. Interestingly, relative levels of SRFR1 in *sum1-1* are comparatively reduced compared to wild-type plants (Fig. 38c). We further demonstrate that in *sum1-1* plants, SRFR1 is targeted for degradation by the proteasome-mediated pathway. Addition of the proteasome-inhibitor MG132 stabilizes SRFR1 in *sum1-1* plants (Fig. 37c). Considering our previous observation that immunity is enhanced in the *sum1-1* plants, our results suggest that AtSUMO1 is involved in the stability of endogenous SRFR1.

Molecular mechanisms underlying legume-powdery mildew interactions

Dr. Divya Chandran

Principal Investigator



Group members

Raghavendra Aminedi
Naini Burman
Gunjan Sharma
Megha Gupta
Arunima Gupta
Akriti Sharma
Varsha Gupta
Divya Saxena
Sujeet Powar

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 novel targets for biotechnological intervention that will help develop effective and eco-friendly disease control measures in important crop legumes. Interactions between the pea powdery mildew *Erysiphe pisi* and its legume hosts are studied to address three major objectives: (1) identification of plant genes that promote successful fungal colonization, (2) identification of plant defense responses that restrict fungal growth preferably without an associated yield penalty, and (3) identification of fungal molecules that promote disease.

Powdery mildew is a devastating plant disease that causes yield losses of 25-60% in important food legumes, including pea. This disease is caused by a biotrophic fungal pathogen, which can infect and multiply only on living host plants. Fungicides that are traditionally used to control the disease are neither cost-effective nor environmentally friendly. Therefore, the broad goal of this programme is to study the interplay between the powdery mildew fungus *Erysiphe pisi* and its host plants *Medicago truncatula* and pea to develop effective and sustainable disease management solutions in legumes (Fig. 38).

One of the major objectives of the program is to identify host genes and pathways responsible for powdery mildew resistance or susceptibility. Another objective is to identify fungal virulence proteins and their targets within the host that can modulate plant defense signaling. We anticipate that targeting a combination of factors would result in dramatically reduced pathogen growth and contribute to durable resistance that is less likely to be rapidly overcome by pathogen counter-evolution.

Powdery mildew fungi secrete small proteins known as 'effectors' into host cells. These effectors generally help the fungus suppress plant defenses and cause disease. Remarkably, plants in turn have evolved resistance proteins that can recognize these effectors and trigger defense signaling. Therefore, identification of fungal effectors and their targets within host plant cells is critical for uncovering mechanisms of pathogenicity as well as discovering novel aspects of plant immunity. Effectors are secreted mainly through two fungal structures. One structure is formed on the plant surface and aids in the penetration of the plant cell wall, and

the second is formed within the cell wall and serves as a feeding structure, helping the fungus draw nutrients and water from the plant. We previously predicted 308 effector candidates from *E. pisi* genes preferentially expressed in the feeding structure. To determine whether these effectors are expressed at particular stages of the fungal life cycle, we studied their expression patterns over the course of infection. Overall, two broad patterns of expression were observed. One set of effectors showed high expression during penetration and primary feeding structure formation. Another set showed high expression at all time points of infection from penetration to fungal reproduction. We further characterized one effector candidate from each set to elucidate their role in legume-powdery mildew interactions. We found that effector A was targeted to the nucleus of the host cell whereas effector B was predicted to localize just outside the plant cell membrane. To explore the functional role of these effectors, we individually knocked down the expression of these genes in the fungus and then tested the ability of these modified fungi to cause disease on pea plants. We found fewer disease symptoms and reduced fungal growth on leaves in which the expression of *effector A* or *B* was reduced. This suggests that effector A and B somehow contribute to the ability of *E. pisi* to cause disease on pea. Effector A belongs to a superfamily of proteins that is associated with the feeding structure in powdery mildew species that infect barley and wheat. These type A effectors have been reported to function as the virulence factors that trigger immunity upon recognition by specific plant resistance proteins. *Effector B* encodes a protein that is conserved across all pathogenic fungi including the powdery mildews. Deletion of similar genes in the rice blast fungus affects penetration and disease development suggesting that this effector may be an important virulence factor. We are currently

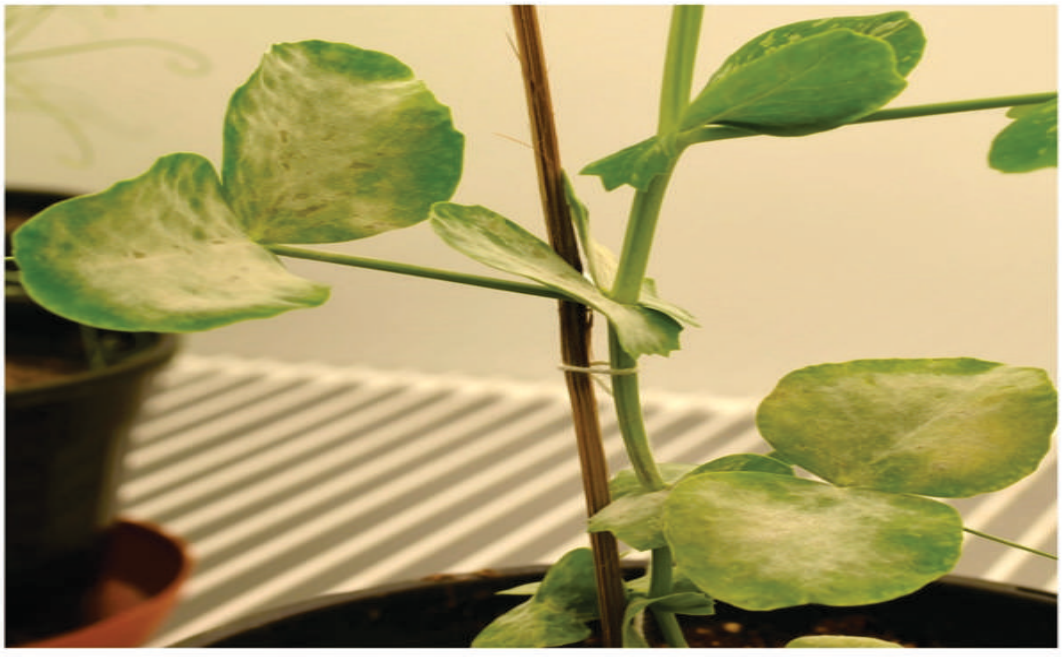


Figure 38: *White powdery mildew disease symptoms on leaves of a susceptible pea plant.*

screening the remaining effector candidates for altered powdery mildew disease phenotypes. In the future, we will identify targets of these effectors inside host cells to understand their mode of action.

E. pisi is a biotrophic fungus that depends entirely on its host plant for nutrients, including sugars. As a consequence, *E. pisi* infection creates an additional sink in plant tissues, which can lead to significant changes in sugar transport and partitioning within the host plant. Sugars can either help or hinder

pathogen growth-they provide nourishment to the growing pathogen and/or act as signaling molecules to induce plant defenses. Therefore, manipulation of sugar transport or signaling may provide an innovative and promising approach to control powdery mildew disease in legumes. We previously reported that the expression of a *Medicago truncatula* sugar transporter (*MtSTP*) was induced rapidly and to a greater extent in a resistant plant compared to a susceptible one in response to *E. pisi* infection. A number of studies have linked STP function with disease resistance. For example, both bacterial and fungal pathogens were found to induce the expression and activity of an STP transporter in *Arabidopsis*. When this gene was overexpressed in *Arabidopsis*, more glucose was transported into the plant cell, away from the compartment harboring the pathogen, and plants were more resistant to the pathogen. It was hypothesized that increased transport of glucose into the plant cell may have limited the amount of glucose available to the pathogen resulting in reduced growth. In a more recent study, a naturally occurring variant form of this transporter was found to be responsible for partial resistance against three wheat pathogens, including powdery mildew. The variant form, which differs from the conserved wild type by two amino acids, was able to block glucose uptake activity of the wild type through direct physical association. In this study, it was hypothesized that changes in hexose to sucrose ratios in the plant cell, brought about by the activity of the variant form of STP, was somehow able to trigger plant defenses and restrict pathogen growth. Taken together, these studies suggest that both enhanced and reduced glucose uptake, facilitated by the different forms of STP, contribute to disease resistance. However, the molecular basis of this resistance has not been explored. We, therefore, set out to uncover the role of MtSTP in the context of legume-powdery mildew interactions. Using a fluorescent tag, we showed that MtSTP localizes to the plant cell plasma membrane. In addition, we demonstrated that MtSTP is a broad range hexose sugar transporter as it

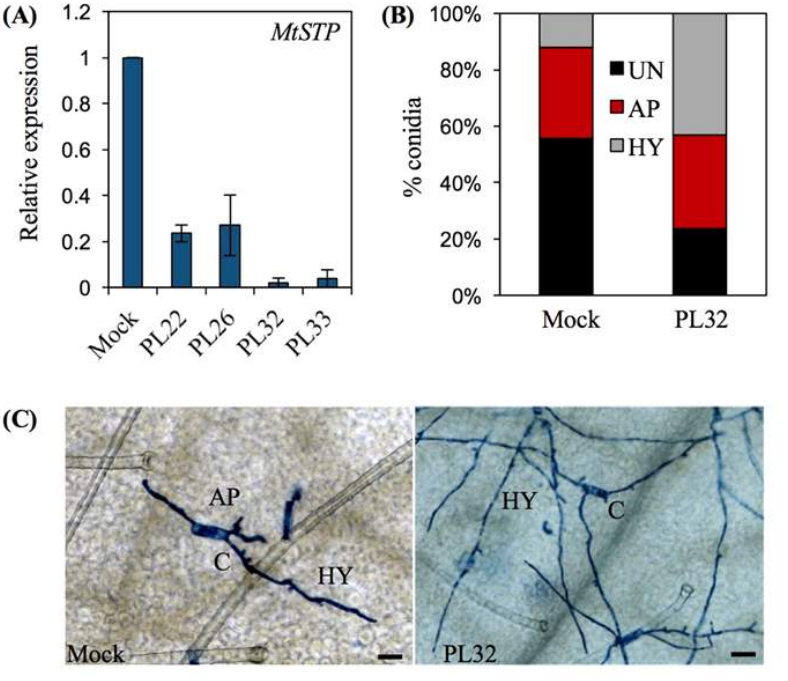


Figure 39: *MtSTP may contribute to powdery mildew resistance. (A) Relative expression of MtSTP in mock and MtSTP-silenced lines (PLs) (B) E. pisi growth stage quantification at 3 dpi. UN, Ungerminated conidia, AP, Appressorium, HY, Hyphae, C, Conidium, dpi, days post inoculation (C) Microscopic view of trypan blue-stained fungal structures. Scale bar= 50 µm.*

was able to rescue the growth of a yeast sugar transporter mutant on different hexose sugar containing media. To check the impact of MtSTP on powdery mildew growth, we silenced *MtSTP* expression in leaves of a moderately resistant *M. truncatula* plant and then infected the plants with *E. pisi*. Leaves in which *MtSTP* expression was reduced by 90% supported greater fungal growth compared to control leaves, suggesting that MtSTP function contributes to powdery mildew resistance. This result is consistent with previous studies conducted in *Arabidopsis* where *stp* mutants showed enhanced susceptibility to bacterial and fungal pathogens. Sequence alignment of STP proteins from wheat (variant and wild type), *Arabidopsis* and *M. truncatula* showed that MtSTP contains only the wild type residues. When we converted the wild type MtSTP into the variant form via site-directed mutagenesis, it was no longer able to rescue the growth defect of the yeast hexose transporter mutant on glucose containing media. This implies that the residues conserved in the wild type are critical for the transport function of MtSTP. We are currently employing a transgenic approach to further investigate the role of MtSTP in powdery mildew resistance (Fig. 39).


39

ACADEMIC ACTIVITIES

Academic Activities

PhD Programme in Biotechnology at RCB

Regional Centre for Biotechnology has a PhD programme in Biotechnology for students who have interest in working at the interface of multiple disciplines in the areas related (but not limited) to structural, systems, synthetic and chemical biology, analysis of complex diseases for identification of intervention points, development of knowledge-based drug discovery strategies, plant biotechnology, enzyme engineering, fermentation among others. After assuming the status of an 'Institution of National Importance', RCB is now offering doctoral degrees on its own. Presently, there are 27 students registered with RCB for PhD in Biotechnology.

PhD Programme at Recognized Centres

RCB has approved academic recognition of various eminent institutions as per Clause 10 (1) f of the RCB Act and as per the RCB Ordinance No. 19 for conducting various programmes and activities, one of which is registering students for the PhD programme under Regional Centre for Biotechnology. The institutions that have received academic recognition with RCB so far are:

- Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad
- Center of Innovative and Applied Bioprocessing (CIAB), Mohali
- National Institute of Animal Biotechnology (NIAB), Hyderabad
- National Agri-Food Biotechnology Institute (NABI), Mohali

These institutions will register PhD students with RCB from 2018.

PhD (integrated) degree (MSc-PhD) Program

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. The course curriculum for this programme has been finalized. Applications from Indian candidates have been received and the screening process is on. Application for this programme are still open for the international candidates.

Semester and Summer Research Training Programmes at RCB

RCB offers research training programmes to post-graduate students of science from various universities/institutions/colleges of repute to carry out their project work towards partial fulfilment of their post-graduate degrees. Selection is based on the strength of resume and evaluation of write-up on their research interests. Selected candidates undergo research training under the mentorship of members of RCB faculty. They learn to carry out their own research projects in collaboration with other group members. Trainees get a realistic experience of several facets of conducting modern biological research and embarking on a research career. The training programmes range from six-months duration (January to July) to two-month Summer internship. This year 9 research trainees joined for six-months duration.

Seminars by Visiting Scientists

Date	Speaker	Title
March 5, 2018	**Dr. T. Oikawa** TEM Application Specialist JEOL Limited, Japan	Recent advancements in Cryo–TEM and overview of 200kV JEOL's TEM Model JEM-2200FS
February 27, 2018	**James Kim** Asia Pacific Regional Sales Manager, CES Department, Macrogen, South Korea	Introduction to Macrogen Sequencing Services, in association with Bencos Research Solutions: paving the way for a simplified research environment in India
February 19, 2018	**Dr. Roop Malik** Dept. of Biological Sciences TIFR, Mumbai	Who Let the Fat Out?
February 6, 2018	**Dr. Teiichi Tanimura** Nagoya University, Japan	Feeding decision making in *Drosophila*
January 24, 2018	**Dr. Ramesh Kumar** DUKE-NUS, Singapore	High throughput screen identified synthetic drug compound lethal to mutant P53
January 23, 2018	**Nandini Verma** Department of Molecular Cell Biology Weizmann Institute of Science, Israel	Targeting progression and drug resistance in Triple Negative Breast Cancer
October 26, 2017	**Dr. Gaurav Ahuja** Centre for Molecular Medicine, and Max Planck Institute for Biology of Ageing, Cologne, Germany	Lysosphingolipid imbalance drives ageing in the heart
September 8, 2017	**Prateek Tripathi, PhD** Research Associate 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**	MicroRNA pathways and their role in aging and neurodegeneration in Drosophila.
June 14, 2017	**Dr. Geeta Ram**	The role of staphylococcal pathogenicity islands (SaPIs) in the adaptation and virulence of Staphylococcus aureus
May 31, 2017	**Dr. Nidhi Adlakha**	Path to Product-Development of microbial cell factories for innovative bioproduction

Date	Speaker	Title
May 25, 2017	**Dr. Tarun Jain** Computational Aspects of Drug Discovery in Both Academia and Industry	Application of Computational Techniques in Drug Discovery
April 27, 2017	**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	**Malay Patra** Department of Chemistry, University of Zurich, Zurich, Switzerland	Glyco-Conjugation Strategy for Targeted Delivery of Platinum Anticancer Drugs
April 07, 2017	**Sabari Sankar Thirupathy, PhD** University of Wisconsin-Madison	Genomic instability at the crossroads of replication and transcription

Scientific Events at RCB

The RCB Bio-imaging School 2018

The first RCB Bioimaging School was conducted during March 19 – 24, 2018. The school highlighted popular imaging systems that have been extensively used in biology and biomedicine. Twenty-one participants from across the spectrum of research scholars, postdoctoral fellows and faculty were selected for the school from across India. The instructors and speakers included experts from prestigious academic research institutions and universities in India and Japan, including the Indian Institute of Science, Jawaharlal Nehru University, the Biomedical Research Institute of the National Institute of Advanced Industrial Science and Technology, Japan and the Regional Centre for Biotechnology. The school was organized by Dr. Sivaram V. S. Mylavarapu and Dr. Sam J. Mathew (faculty members) and Mr. Suraj Tewari (Technical Assistant) of RCB.

The speakers delivered expert lectures on the various imaging technologies and their applications, with a focus on their ongoing research. Application experts from various global leaders in bioimaging instrumentation including Leica Microsystems, Olympus, Nikon Corporation and Carl Zeiss also participated. The approaches showcased and discussed included widefield, confocal and super resolution fluorescence imaging, laser capture microdissection microscopy, fluorescence correlation spectroscopy and atomic force microscopy. The participants were imparted theoretical knowledge and practical training to showcase the power and versatility of these imaging methods. Some modules on quantitative experimental data analysis using the latest imaging software analysis tools were also demonstrated. The participants left the imaging school with a wide appreciation of the various biomedical imaging methods, as well a deeper understanding of certain applications of state-of-the-art optical imaging technologies in use across the globe. These imaging technologies have enabled path-breaking discoveries in basic biology and continue to revolutionize advances in biotechnology and medicine.

RCB-AIST Mini-symposium

A mini-symposium was organized on March 24, 2018 at the end of the 'First RCB Bioimaging School'. The mini symposium was designed to facilitate scientific exchange between researchers of the Biomedical Research Institute, AIST Japan and RCB, Faridabad to facilitate scientific collaboration. Distinguished scientists as well as young researchers from both the BRI and RCB presented short research talks highlighting their ongoing research, and was attended by an audience of researchers from RCB as well as the participants of the RCB Bioimaging School.

National Science Day

India celebrates February 28 every year as the 'National Science Day' to pay tribute to the *Raman Effect* of the Nobel Laureate Sir C.V. Raman. To commemorate this day, Regional Centre for Biotechnology, Faridabad conducted a scientific quiz and a debate competition for undergraduate science students from various colleges in and around Faridabad. Prof. Sudhanshu Vrati, the Executive Director, RCB opened the day with his welcome address. Dr. Vrati highlighted the importance of Science and Biotechnology in their daily lives and encouraged the college students to take up science as a career.

About 100 students from different colleges visited RCB and participated in the debate and quiz competition. As part of the debate competition, they expressed their views on the pros and cons of genome editing. In addition, a scientific communication session was held, where a student from each lab presented a non-technical summary of the ongoing research in their lab. The day ended with a tour of the state-of-the-art research facilities such as microscopy, plant-tissue culture, X-ray diffraction and flow-cytometry. This session exposed students to a scientific laboratory environment and gave them a glimpse of the cutting-edge research being conducted at RCB. The programme ended with distribution of certificates and prizes. Overall, the event was successful in inspiring the younger generation and kindling their curiosity towards science.

RCB Day 2018

In 2016, RCB was ordained with the status of an "Institution of National Importance" through an Act of the Parliament. It was brought into effect by a Gazette notification on 1st March, 2017. To commemorate this momentous occasion, 1st March has been adopted as the RCB Day.

The first RCB Day was observed in 2018 and was celebrated with a blend of scientific and cultural events. The day began with final year PhD students presenting their research work in front of a panel of judges from various institutes in Delhi-NCR. The presentations were followed by a welcome address by the Executive Director, Dr. Sudhanshu Vrati. The director then invited the eminent scientist, Padma Bhushan Prof. Govindarajan Padmanaban to enrich the audience with his talk titled 'Biotechnology Innovation in Health Sector'. His talk gave insights into the discoveries made by his group on finding new drug targets for malaria. He emphasised the need to develop innovative biotechnological tools to combat newer biological threats. The Executive Director felicitated Prof. Padmanaban with a memorial plaque and shawl. Prof. Padmanaban handed over certificates and cash prizes to the top three student presenters of the day.

The academic activities were followed by a grand Bharatanatyam recital by Vidushi Gita Chandran, a recipient of the prestigious Padma Shri in 2007 and Sangeet Natak Akademi Puraskar in 2016. She and her troupe were felicitated by Prof. G. Padmanaban. The enthusing evening of the first RCB Day celebration came to a conclusion with a gala dinner over which, the students interacted with the eminent personalities.

Lectures delivered/ Conferences attended/ Visits abroad

Dr. Deepak T. Nair

1. Delivered an invited talk titled "Structural Biology of DNA polymerases" at the "DST INSPIRE Science Camp" organized for outstanding school students by NIST, Behrampur, March 20-24, 2018.
2. Delivered an invited talk titled "Time-resolved crystallography reveals snapshots of the DNA synthesis reaction" at the "42nd Annual Meeting of the Indian Biophysical Society Meeting" held at IISER-Pune, March 9-11, 2018.
3. Delivered an invited talk titled "Structural Studies on Molecular Determinants of Genomic Integrity" at the "Indo-US Conference on Transcription, Chromatin Structure, DNA Repair and Genomic Stability" at IISc, Bangalore, March 6-10, 2018.
4. Delivered an invited talk titled "ESRF India Collaboration and crystallography" at the "Discussion Meeting on Synchrotron Science" at Saha Institute of Nuclear Physics, Kolkata, December 13-15, 2017.
5. Delivered an invited talk titled "Mechanism of formation of a toroid around DNA by the Mismatch Sensor Protein" at the "National Conference on Protein Structure and Dynamics in Health and Agriculture" held at Jamia Milia Islamia, November 3-4, 2017.
6. Delivered an invited talk titled "Birth of the Phosphodiester bond" at the conference titled "Structure across Scales" held at NCBS, Bangalore, October 7-8, 2017.
7. 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.
8. Attended "Guha Research Conference", 2017 held at Kumarakom, Kerala, December 2-6, 2017.
9. Visited Grenoble (France) to collect data from macromolecular crystals at the European Synchrotron Radiation Facility, during July 22-25, 2017.

Dr. Chittur V. Srikanth

1. Delivered a talk titled "My Journey with *Salmonella* and SUMO in India: an ongoing quest to leave a trail" at the "Young Investigator Meeting 2018" held at Thiruvananthapuram, Kerala, March 5-8, 2018.
2. Attended Global Forum on TB Vaccines, held at New Delhi, February 20-23, 2018.
3. Delivered an invited talk titled "Gut Pathogen Salmonella remodels host-SUMO landscapes to gain intracellular survival", at SASTRA University Tanjavur, February 12, 2018.

Dr. Deepti Jain

1. Discussion on "Women-in-science – Listening session" organized by the Wellcome Trust/DBT Indian Alliance and US Embassy on November 16, 2017 at ICGEB, New Delhi
2. Delivered an invited talk titled " Regulation of flagellar and biofilm genes in *Pseudomonas aeruginosa*. " at the "Marie Curie Sesquicentennial Conference" organized by the Indian National Young Academy of Science from November 5-7, 2017 at JNU, New Delhi.

3. 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.
4. Attended the "National CryoEM Facility Inauguration Meeting" from January 24-25, 2018 NCBS, Bangalore.
5. Poster presentation at the "India International Science Festival – Women Scientist Entrepreneur Conclave" organized by DBT from October 15-16, 2017, Chennai.
6. Attended the DBT-EMBL conference on October 12-13, 2017 in New Delhi for exploring collaborations with EMBL in the field of Structural Biology, Imaging and Bioinformatics.

Dr. Vengadesan Krishnan

1. Delivered an invited talk titled "Structural insights into the probiotic pilus formation from synchrotron radiation" in the "Discussion Meeting on Synchrotron Science" held at Saha Institute of Nuclear Physics, Kolkata, December 13-15, 2017.
2. 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.
3. Participated and presented work on "Visualizing the Architecture of Pili in a Probiotic Bacterium" in the "EMBO Practical Course, Cryo-Electron Microscopy and 3-Dimensional Image Processing of macromolecular assemblies and cellular tomography" held at IIT Delhi, March 18-29, 2018.
4. Attended and presented report in 32nd DeLCON Nodal Officer's Meeting held at NCCS, Pune, October 23-24, 2017.
5. Participated and member of organizing/advisory committee in 'National Workshop on Strengthening Open Access (OA) Initiatives in India' at NBRC, Manesar, June 23, 2017.
6. Visited MX beamlines at European Synchrotron Radiation Facility (ESRF), France, May 11-16, 2017 under DBT-ESRF partnership programme.

Dr. Kanchan Bhardwaj

1. Attended "Workshop on chikungunya vaccines; Challenges, opportunities and possibilities" at New Delhi, February 5-6, 2018.

Dr. Sudhanshu Vrati

1. Delivered an invited lecture titled "Development of a rotavirus vaccine: The India Story" at the "VIROCON 2017 Annual Conference" held at NITTE University, Mangalore, December 7-9, 2017.
2. Delivered an invited lecture titled "Mosquito-borne virus infection: development of vaccine candidate" at the "International Vaccine Conference" held at ICGEB, New Delhi, November 27-29, 2017.
3. Delivered an invited talk titled "Development of a rotavirus vaccine: The India Story" at the Annual Conference of the Society of Biological Chemists", at JNU, New Delhi, November 17, 2017
4. Delivered an invited lecture titled "Development of a rotavirus vaccine: The India Story" held at IP University, New Delhi, November 8, 2017.
5. Delivered an invited lecture titled "Role of host cell proteins in Japanese encephalitis virus replication" at South Asian University, New Delhi, September 13, 2017.

6. Delivered keynote lecture titled "Development of a rotavirus vaccine: The India Story" at IVRI, Mukteswar, June 10-13, 2017.
7. Delivered inaugural day lecture titled "Development of a rotavirus vaccine: The India Story" in "Manipal Research colloquium", organized by Manipal University, April 3-4, 2017.
8. Participated in DBT-Ramalingaswami Conclave, organized by IBSD, Imphal, Manipur, August, 29-31, 2017.
9. Participated in the Nobel Prize India Series 2018, Goa, February, 1-2, 2018.

Dr. Tushar Kanti Maiti

1. Delivered an invited talk on "Protein redox modification dictates Parkinson's disease pathology" at the "International Conference on Trends in Biochemical and Biomedical Research: Advances and Challenges" organized by Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi, February 13-15, 2018.

Dr. Sam J. Mathew

1. Organized the 1st RCB Bioimaging School at RCB, March 19-24, 2018.
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. Attended the '7th Annual Fellows Meeting' organized by the Wellcome Trust-DBT India Alliance, Hyderabad and presented a poster entitled 'Myosin Heavy Chain-Embryonic is a Key Regulator of Skeletal Muscle Differentiation During Mammalian Embryonic and Fetal Development', May 18-20, 2017.
4. 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, "The role of developmental myosin heavy chains in skeletal muscle development, regeneration, homeostasis and disease", from 24.05.2017-22.06.2017 and 01/11/2017 to 31/01/2018.

Dr. Geetanjali Chawla

1. Delivered a talk titled "Let-7-Complex microRNAs modulate lifespan and neuronal integrity in *Drosophila*" at the "2nd RCB-AIST mini-symposium" held at RCB, Faridabad, March 24, 2018.
2. Attended "Workshop on Research Methodology" organized by Wellcome-DBT India Alliance held at THSTI, Faridabad, March 14-15, 2018.
3. Attended the "3rd Biennial Indian Drosophila Research Conference 2017", held at IISER Bhopal, December 6-9, 2017.

Dr. Sivaram V.S. Mylavarapu

1. Delivered an invited lecture titled "Understanding the Biogenesis and Function of Tunneling Nanotubes" at the "International Congress of Cell Biology" organized by the CSIR-Centre for Cellular and Molecular Biology, January 27-31, 2018.
2. Delivered an invited lecture titled "Understanding the Biogenesis of Tunneling Nanotubes" at the "Current Trends in Intracellular Transport and Molecular Motors" meeting organized by Indian Institute of Technology Bombay and the Tata Institute of Fundamental Research Mumbai, December 21-23, 2017.

3. Delivered invited lecture titled 'Motoring through Mitosis' at the symposium on New Directions in Cell Signaling, organized by Amity University Haryana, April 21, 2017.
4. Attended the "Second Indian *C. elegans* Meeting" held at the National Institute of Immunology, New Delhi, February 23-26, 2018.
5. Organized the 1st RCB Bioimaging School at RCB, March 19-24, 2018.

Dr. Saikat Bhattacharjee

1. Delivered a talk titled "Inositol-phosphates connect to innate immune signaling in *Arabidopsis thaliana-Pseudomonas syringae* interactions" at CDFD, Hyderabad, December 8, 2017.
2. Delivered a talk titled "Plant Immune modulators and their strategic coupling to signalling pathways during effector-triggered immunity" at Sea6Energy Pvt. Ltd., Bengaluru, on 3rd November, 2017.
3. 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, M.P., October 27–29, 2017.

Dr. Divya Chandran

1. Delivered an invited lecture titled "Resolving plant-pathogen interactions using laser microdissection" at the RCB Bioimaging School, RCB, Faridabad, March 19-24, 2018.
2. Delivered an invited lecture titled "Understanding the legume-powdery mildew interaction: a systems level approach" at the "International Congress of Cell Biology", Hyderabad, January 27-31, 2018.
3. Delivered an invited lecture titled "Genomics-enabled insights into legume-powdery mildew interactions" at the "Molecular intricacies of Plant-Microbe Interactions" conference, Indira Gandhi National Tribal University, Amarkantak, October 27-29, 2017.
4. Presented a poster titled "Transcriptome profiling of enriched pea powdery mildew haustoria reveals novel candidate effectors" at the "Women Scientists and Entrepreneur's Conclave", Anna University, Chennai, October 15-16, 2017.
5. 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 October 4, 2017.
6. Delivered invited lecture titled 'Genomics-enabled insights into legume-powdery mildew interactions' at the Genomics Analysis and Technology Conference, Bhubaneswar, 8-9 September 2017.

Dr. Pinky Kain

1. Delivered an invited talk titled "Eat it less and eat it right- Understanding modulation of taste behavior by dietary high salt in *Drosophila melanogaster*" at Indian *Drosophila* Annual conference, organized by IISER Bhopal, December 6-9, 2017.
2. Attended the 'Annual Fellows Meeting', Hyderabad by Wellcome Trust/DBT India Alliance, May 18-20, 2017.

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

Dr. Deepak T. Nair

1. Member, Guha Research Conference
2. Member, Indian Crystallography Association
3. Member, Society of Biological Chemists
4. Member, Indian Biophysical Society
5. Member, Board of Studies, Regional Centre for Biotechnology
6. Member, Academic Committee, Regional Centre for Biotechnology
7. Member of the Expert Committee to review Indo-Japan collaborative projects, Department of Biotechnology
8. Member of the Standing Consultative Committee of Young Scientists, Ministry of Science & Technology constituted by the Office of the Principal Scientific Advisor.
9. Member of the Screening Committee (Membrane Structural Biology), Department of Biotechnology
10. Invited Member of the Biochemistry, Biophysics, Microbiology & Molecular Biology Program Advisory Committee of the Science & Engineering Research Board.

Chittur V. Srikanth

1. Member, American Society for Microbiology
2. Member, Infectious Disease Biology task force of Department of Biotechnology

Dr. Deepti Jain

1. Member, Indian Crystallography Association
2. Member, Society of Biological Chemists
3. Member, Electron Microscopy Society

Dr. Vengadesan Krishnan

1. Member, Indian Crystallographic Association
2. Member, Indian Biophysical Society
3. Member, International Union of Crystallography

Dr. Prasenjit Guchhait

1. *Ad-Hoc* reviewer for Journals: Cellular Physiology and Biochemistry, Integrative Biology, Scientific Reports, Annal Hematology, Journal of Immigrant and Minority Health, Thrombosis Research
2. Member, Editorial Board for journals: Annals of Clinical and Experimental biology, Austin Hematology and Cardiology: Open Access

Dr. Sudhanshu Vrati

1. Life Member, Indian Society for Cell Biology
2. Life Member, Society of Biological Chemists, India
3. Life Member, Association of Microbiologist of India
4. Life Member, Indian Immunology Society
5. Life Member, Indian Virology Society

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

Dr. Geetanjali Chawla

1. *Ad hoc* reviewer, Cell biology and Toxicology
2. *Ad hoc* reviewer, Parasites and Vectors

Dr. Sivaram V. S. Mylavarapu

6. Life Member, Indian Society for Cell Biology
7. Life Member, Society of Biological Chemists, India
8. Reviewer, Scientific Reports

Dr. Saikat Bhattacharjee

1. Review Editor, Frontiers in Plant Science
2. Expert Reviewer, Plant-Microbe Interactions Call, DBT, Govt. of India

Dr. Divya Chandran

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

Dr. Pinky Kain

1. Associate Editor, Journal of Experimental Neuroscience, 2018

Distinctions, Honours and Awards

Dr. Deepak T. Nair

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

Dr. Chittur V. Srikanth

1. Awarded DBT Wellcome Trust Indian Alliance intermediate Fellowship (2012-17)

Dr. Deepti Jain

1. Best Poster Award at the India International Science Festival – Women Scientist Entrepreneur Conclave, Organized by DBT, October 15-16, 2017 Chennai
2. SERB Young Investigator Award, Department of Science and Technology, Government of India, March 2016-2019.

Dr. Sudhanshu Vrati

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

Dr. Sam J. Mathew

1. Invited to participate as a mentor at the Wellcome Trust-DBT India Alliance Science Communication workshop, New Delhi, 7-8 September, 2017.
2. Featured in the India Alliance newsletter "News & Views", Issue 17, as India Alliance Fellow in Spotlight, September 2017.
3. Fellowship supervisor of Dr. Masum Saini, who has been recommended for a Wellcome Trust-DBT India Alliance Early Career Fellowship in June 2017.
4. Chaired Session of scientific presentations at the '7th Annual Fellows Meeting' organized by the Wellcome Trust-DBT India Alliance, Hyderabad, May 18-20, 2017.

Dr. Geetanjali Chawla

1. Awarded Wellcome Trust-DBT India Alliance Intermediate fellowship (2018-2022)
2. Awarded Ramalingaswami fellowship, DBT (2016-2017) (Relinquished)

Publications

1. Nirwal S, Kulkarni DS, Sharma A, Rao DN, **Nair DT*** (2018). Mechanism of formation of a toroid around DNA by the mismatch sensor protein. *Nucleic Acids Res*, 46: 256.

2. Salunke DM* and **Nair DT*** (2017). Macromolecular Structures: Quality Assessment and Biological Interpretation. IUBMB Life, 69: 563.

3. Mustfa SA, Singh M, Suhail A, Mohapatra G, Verma S, Chakravorty D, Rana S, Rampal R, Dhar A, Saha S, Ahuja V and **Srikanth CV*** (2017). SUMOylation pathway alteration coupled with downregulation of SUMO E2 enzyme at mucosal epithelium modulates inflammation in inflammatory bowel disease. Open Biology, 7(6): 170024.

4. **Srikanth CV***, Verma S (2017). Sumoylation as an Integral Mechanism in Bacterial Infection and Disease Progression. *Adv Exp Med Biol*, 963: 389.

5. Naskar T, Faruq M, Banerjee P, Khan M, Midha R, Kumari R, Devasenapathi R, Prajapati B, Sengupta S, **Jain D,** Mukerji M, Singh NC, Sinha S* (2018). Ancestral Variations of the PCDHG Gene Cluster Predispose to Dyslexia in a Multiplex Family. *EBioMedicine*, 28: 168.

6. 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 rhamnosusGG. Acta Crystallographica Section F: Structural Biology Communications, 73: 321.

7. Gurjar BS, Sriharsha TM, Bhasym A, Prabhu S, Puraswani M, Khandelwal P, Saini H, Saini S, Verma AK, Chatterjee P, **Guchhait P**, Bal V, George A, Rath S*, Sahu A, Sharma A, Hari P, Sinha A, Bagga A (2018). Characterization of genetic predisposition and autoantibody profile in atypical hemolytic uremic syndrome. *Immunology, 154: 663.*

8. Tashi T, Reading NS, Wuren T, Zhang X, Moore LG, Hu H, Tang F, Shestakova A, Lorenzo F, Burjanivova T, Koul P, **Guchhait P**, Wittwer CT, 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 (HIF2α) polymorphism lowers hemoglobin concentration in Tibetans. Journal of Molecular Medicine 95(6): 665-670.

9. Pandey AD, Goswami S, Shukla S, Das S, Ghosal S, Pal M, Bandyopadhyay B, Ramachandran V, Basu N, Sood V, Pandey P, Chakrabarti J, **Vrati** S, Banerjee A* (2017). Correlation of altered expression of a long non-coding RNA, NEAT1, in peripheral blood mononuclear cells with dengue disease progression. *J Infect*, 75: 541.

10. Sood V*, Sharma KB, Gupta V, Saha D, Dhapola P, Sharma M, Sen U, Kitajima S, Chowdhury S, Kalia M, **Vrati** S* (2017). ATF3 negatively regulates cellular antiviral signaling and autophagy in the absence of type I interferons. *Sci Rep*, 7: 8789.

11. Madhvi A, Hingane S, Srivastav R, Joshi N, Subramani C, Muthumohan R, Khasa R, Varshney S, Kalia M, **Vrati** S, Surjit M, Ranjith-Kumar CT* (2017). A screen for novel hepatitis C virus RdRp inhibitor identifies a broad-spectrum antiviral compound. *Sci Rep*, 7: 5816.

12. Banerjee A*, Shukla S, Pandey AD, Goswami S, Bandyopadhyay B, Ramachandran V, Das S,

Malhotra A, Agarwal A, Adhikari S, Rahman M, Chatterjee S, Bhattacharya N, Basu N, Pandey P, Sood V, **Vrati** S* (2017). RNA-Seq analysis of peripheral blood mononuclear cells reveals unique transcriptional signatures associated with disease progression in dengue patients. *Transl Res*, 186: 62.

13. Chandola TR, Taneja S, Goyal N, Antony K, Bhatia K, More D, Bhandari N, Cho I, Mohan K, Prasad S, Harshavardhan G, Rao TS, **Vrati** S, Bhan MK* (2017). ROTAVAC® does not interfere with the immune response to childhood vaccines in Indian infants: A randomized placebo controlled trial. *Heliyon*, 3: e00302.

14. Sharma M, Bhattacharyya S, Sharma KB, Chauhan S, Asthana S, Abdin MZ, **Vrati** S*, Kalia M* (2017). Japanese encephalitis virus activates autophagy through XBP1 and ATF6 ER stress sensors in neuronal cells. *J Gen Virol*, 98: 1027.

15. Kumar R, Kumari R, Kumar S, Jangir DK, **Maiti TK*** (2018). Extracellular α synuclein disrupts membrane nanostructure and promotes S-nitrosylation induced neuronal cell death. *Biomacromolecules,* 19: 1129.

16. Kumar P, Bag S, Ghosh TS, Dey P, Dayal M, Saha B, Verma J, Pant A, Saxena S, Desigamani A, Rana P, Kumar D, Sharma NC, Hanpude P, **Maiti TK**, Mukhopadhyay AK, Bhadra RK, Nair GB, Ramamurthy T, Das B* (2018). Molecular Insights into Antimicrobial Resistance Traits of Multidrug Resistant Enteric Pathogens isolated from India. *Sci Rep*, 7: 14468.

17. Hanpude P, Bhattacharya S, Singh AK, **Maiti TK*** (2017). Ubiquitin Recognition of BAP1: Understanding its Enzymatic Function. *Biosci Rep,* 37: BSR20171099.

18. 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*, 9: 230.

19. Saini, M., A. Verma, **Mathew SJ*** (2018). SPRY2 is a novel MET interactor that regulates metastatic potential and differentiation in rhabdomyosarcoma. Cell Death and Disease 9: 237.

20. Yavvari PS, Pal S, Kumar S, Kar A, Awasthi A, Naaz A, Srivastava A, **Bajaj A*** (2017). Injectable, Self-healing Chimeric Catechol-Fe(III) Hydrogel for Localized Combination Cancer Therapy. *ACS Biomaterials Sci & Eng,* 3: 3404.

21. Sreekanth V, Medatwal N, Kumar S, Pal S, Malyla V, Kar A, Bhargava P, Naaz A, Kumar N, Sengupta S, **Bajaj A*** (2017). Tethering of Chemotherapeutic Drug/Imaging Agent to Bile Acid-Phospholipid Increases the Efficacy and Bioavailability with Reduced Hepatotoxicity. *Bioconjugate Chem,* 28: 2942.

22. 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*, Singh R* (2017). The dual mechanism of action of 5-Nitro-1,10-Phenanthroline against *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother,* 61: e00969-17.

23. Kundu S, Bansal S, Muthukumarasamy KM, Sachidanandan C, Motiani R*, **Bajaj A*** (2017). Deciphering the Role of Hydrophobic and Hydrophilic Bile Acids in Angiogenesis using in vitro and in vivo Model Systems. *Med Chem Comm,* 8: 2248.

24. 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, Chowdhury S* (2017). Transcriptional regulation of CDKN1A(p21/CIP1/WAF1) by TRF2 through the REST repressor complex. *Scientific Reports,* 7: 11541.

25. 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.

26. 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.

27. Halane MK, Kim SH, Spears BJ, Garner CM, Rogan CJ, Okafor EC, Su J, **Bhattacharjee S**, Gassmann W*. (2018). The Bacterial Type III-Secreted Protein AvrRps4 is a Bipartite Effector. *PLoS Pathog*, 14: e1006984.

28. Wildermuth MC, Steinwand MA, McRae AG, Jaenisch J and **Chandran D*** (2017). Adapted biotroph manipulation of plant cell ploidy. Annual Review of Phytopathology, 55: 537.

*Corresponding Author

Patent application filed

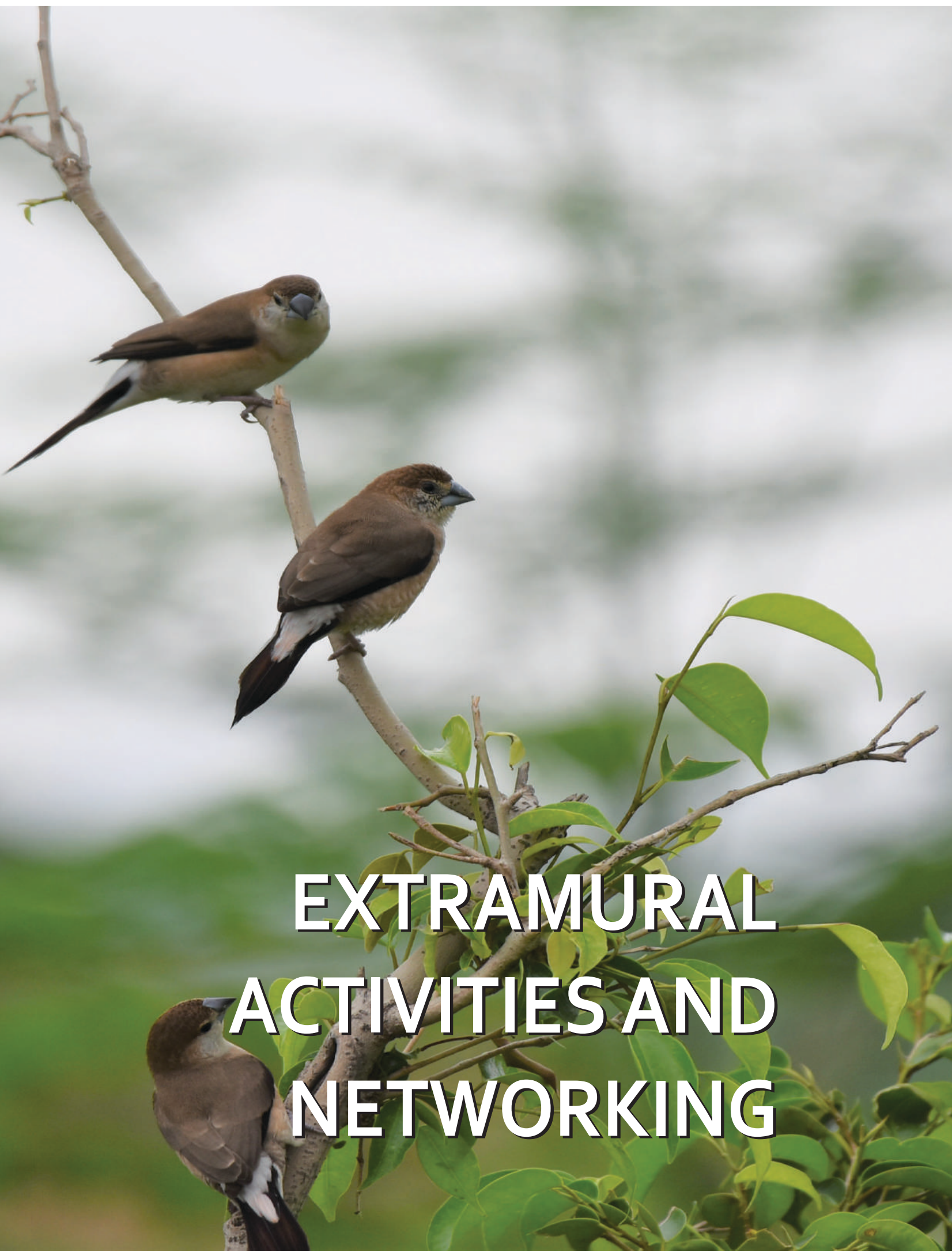
1. Sreekanth Vedagopuram, Sandeep Kumar, Sagar Sengupta, **Avinash Bajaj** (June 21, 2017). Conjugated Anti-proliferative Drug Nano-particles and Process for Preparation thereof 2017 (PCT/IN2017/050253).

Distinguished Lectures

Date	Speaker	Title
March 1, 2018	Prof. G. Padmanaban	Biotechnology innovation in the health sector
October 5, 2017	Prof. Seyed E. Hasnain Jamia Hamdard, New Delhi	Understanding the Making of TB to find ways to unmake it

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 AND NETWORKING

Extramural Activities and Networking

RCB-DAILAB

The Department of Biotechnology (DBT), through the Regional Centre for Biotechnology (RCB) and the National Institute of Advanced Industrial Science & Technology (AIST), through its Biomedical Research Institute (BRI), Japan had entered into a partnership for capacity building initiatives in bio-imaging and biotechnology in the year 2014. This initiative facilitates enhancement of career opportunities for scientists and researchers working in biomedical, clinical, and related areas of biotechnology, complementing 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 facilitates 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 and symposia have been conducted as part of the DAILAB in AIST Japan and at RCB, bringing together experts and students for theoretical and hands on imaging sessions, taking advantage of the facilities and expertise 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.

The 1st RCB Bioimaging School was conducted in March 2018 in partnership with faculty from AIST where young scientists from across the country were trained in the advanced methods and tools used in Bioimaging. RCB and AIST also organized a mini symposium in March 2018 at RCB on contemporary issues in life sciences.

ESRF Access Program

Regional Centre for Biotechnology (RCB) and European Synchrotron Radiation Facility (ESRF) have entered into an agreement concerning the medium-term use of synchrotron for non-propriety research for the benefit of the Indian scientific community as a whole, and notably the structural biology research groups. The program provides access to Indian investigators to high intensity macromolecular crystallography, small angle X-ray scattering experimental stations and the Cryo-Electron Microscopy Facility located in ESRF. The program was flagged off in June, 2017 by the Honourable Minister for Science & Technology, Dr. Harsh Vardhan in the presence of Prof. Sudhanshu Vrati and the then DBT Secretary, Prof. K. VijayRaghavan.

Since the start of this arrangement, researchers from 23 different institutes from all over India have obtained X-ray diffraction, small angle X-ray scattering and Electron Microscopy data for different macromolecules and macromolecular assemblies. The list of institutions are as follows: - Institute of Microbial Technology (Chandigarh), Jawaharlal Nehru University (New Delhi), Institute of Life Sciences (Bhubhaneswar), Institute of Stem Cell & Regenerative Medicine (Bangalore), All India Institute of Medical Sciences (New Delhi), Indian Institute of Science (Bangalore), Poornaprajna Institute of Scientific Research (Bangalore), Regional Centre for Biotechnology (Faridabad), Indian Institute of Science Education & Research-Pune, Indian Institute of Technology-Delhi, Indian Institute of Technology-Roorkee, Indian Institute of Technology-Kharagpur, National Centre for Cell Sciences (Pune), Indian Institute of Science Education & Research-Thiruvananthapuram, Central Drug Research Institute (Lucknow), Saha Institute of Nuclear Physics (Kolkata), National Institute of Mental Health & Neurosciences (Bangalore), National Institute of Science Education & Research (Bhubhaneswar), CSIR-Institute of Genomics & Integrative Biology (New Delhi), CSIR-Central Leather Research Institute (Chennai), University of Madras (Chennai), International Centre for Genetic Engineering and Biotechnology (New Delhi) & Indian Institute of Technology-Bombay (Mumbai). The access to ESRF has helped Indian scientists to obtain data that will aid formulation of innovative solutions to problems faced by the nation in public health, agriculture and environmental issues.

Extramural Funding

S. No.	Investigator	Project	Funding Agency	Grant Amount (Rs.)	Duration
1.	Dr. Deepak T. Nair	Molecular Interactions critical for DNA Mismatch Repair	Science & Engineering Research Board-DST	59.6 lakh	2017-20
2.	Dr. Deepak T. Nair	Access to Macromolecular Crytsallography Beamlines of ESRF, France	Department of Biotechnology	1922.6 lakh	2017-20
3.	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 lakh	2016-19
4.	Dr. Deepak T. Nair	Big Data Initiatives in Biology & Astronomy	National Knowledge Network	150 lakh	2016-19
5.	Dr. Deepak T. Nair	Mechanism of mutagenic & translesion DNA synthesis by a mycobacterial Y-family DNA polymerase	Science & Engineering Research Board-DST	59.3 lakh	2015-18
6.	Dr. Deepak T. Nair	Effect of N^2-Adducts of Deoxyguanosine on DNA Synthesis by Replicative and Translesion DNA Polymerases	Department of Biotechnology	19.9 lakh	2015-18
7.	Dr. C.V. Srikanth	Investigations into structural organization and curvature-dependent membrane binding of alpha-synuclein	DBT-North-East Twinning grant	17.2 lakh	2017-19
8.	Dr. C.V. Srikanth	Studies on epigenetic alterations during *Salmonella* infection and their long term implications	Science & Engineering Research Board - DST	48.0 lakh	2017-19
9.	Dr. C.V. Srikanth	Understanding *Salmonella typhimurium* mediated alterations in host SUMOylation: implications in infection inflammation	Wellcome Trust-DBT India Alliance Intermediate Fellowship	328 lakh	2012-17
10.	Dr. Deepti Jain	Structure and mechanism of FleQ, master regulator of transcription of flagellar and biofilm genes in *Pseudomonas aeruginosa*	Department of Biotechnology	68.1 lakh	2018-21

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	34.8 lakh	2016-19
12.	Dr. Vengadesan Krishnan	Structural studies on pilus proteins from *Lactobacillus ruminis*	Department of Biotechnology	44.5 lakh	2018-21
13.	Dr. Vengadesan Krishnan	Structural investigations of surface nano scale assembly in a gut bacterium	Department of Biotechnology	70 lakh	2014-17
14.	Dr. Prasenjit Guchhait	Mechanism of rapid propagation of dengue virus during infection (jointly with AIIMS and THSTI)	Department of Biotechnology	Total grant: 100 lakh Grant for RCB: 46 lakh	2018-21
15.	Dr. Prasenjit Guchhait	Investigating the mechanism of two anti-platelet drugs, RX101 and RX103	RythRx	USD 10000	2017-18
16.	Dr. Kanchan Bhardwaj	Metagenome sequence analysis of the distal gut virome in healthy Indian adults	Bio-CARe, Department of Biotechnology	58.2 lakh	2017-20
17.	Dr. Tushar Kanti Maiti	A "bench to bedside" model for clinical and translational science between academic research institutes and hospitals focused on fetal growth restriction and preterm birth (jointly with THSTI and Gurgaon General Hospital)	Department of Biotechnology	682 lakh Grant for RCB: 23.1 lakh	2017-21
18.	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)	Department of Biotechnology-BMG Foundation	Total grant: 134.2 lakh Grant for RCB: 61.3 lakh	2016-17

S. No.	Investigator	Project	Funding Agency	Grant Amount (Rs.)	Duration
19.	Dr. Tushar Kanti Maiti	Targeting ubiquitin proteasome system for the anticancer drug development: A peptoid based inhibitor design, synthesis and evaluation	Department of Biotechnology	24.9 lakh	2015-18
20.	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: 4885 lakh Grant for RCB: 613 lakh	2014-19
21.	Dr. Masum Saini (Fellowship supervisor: Dr. Sam J. Mathew)	Role of Sprouty2 as a modulator of Met signaling during mammalian skeletal muscle development, regeneration, and disease	Wellcome Trust-DBT India Alliance Early Career Fellowship	167 lakh	2018-23
22.	Dr. Sam J. Mathew	The Role of Transducin-like Enhancer of Split 3 (TLE3) in Regulating Myogenesis	Science & Engineering Research Board - DST	60 lakh	2017-20
23.	Dr. Sam J. Mathew	The role of MET-CBL signaling in Rhabdomyosarcoma	DBT Cancer grant Pilot Project for Young Investigators	24 lakh	2015-18
24.	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 lakh	2014-19
25.	Dr. Geetanjali Chawla	Post-transcriptional regulators of aging and dietary restriction	Wellcome-DBT India Alliance Intermediate fellowship	359 lakh	2018-22
26.	Dr. Avinash Bajaj	Spatiotemporal targeting of multiple pathway using engineered polymer gatekeepers in porous nanomaterials for cancer combination therapy	DST (for Indo-Korean Grant)	60.8 lakh	2018-21
27.	Dr. Avinash Bajaj	Temporal targeting of siRNA therapeutics to the gastrointestinal tract (GIT) using chimeric nanogels	Department of Biotechnology	84.3 lakh	2017-20
28.	Dr. Avinash Bajaj	Development of biocompatible surfaces for ESKAPE pathogens	Department of Biotechnology	41.3 lakh	2017-20

S. No.	Investigator	Project	Funding Agency	Grant Amount (Rs.)	Duration
29.	Dr. Avinash Bajaj	Molecular engineering of low molecular weight injectable hydrogels with sustained drug release for cancer therapy	Department of Biotechnology	42.6 lakh	2016-19
30.	Dr. Avinash Bajaj	Engineering of self-assembled lipidated nanoparticles for cancer combination therapy	Department of Biotechnology	47.7 lakh	2016-19
31.	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	50.1 lakh	2015-18
32.	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	29.4 lakh	2015-20
33.	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 lakh	2017-20
34.	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)	43.2 lakh	2016-19
35.	Dr. Divya Chandran	Identification of novel regulators and nodes of response mediating powdery mildew sporulation on legumes	Science & Engineering Research Board - DST	39.1 lakh	2017-20
36.	Dr. Pinky Kain	Understanding the taste and its modulation using *Drosophila melanogaster* as a model system	Wellcome trust DBT Alliance intermediate fellowship	350 lakh	2016-21

INFRASTRUCTURE
AND TECHNICAL SUPPORT

Infrastructure and Technical Support

Laboratory Infrastructure

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

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

chemiluminescence imager. DAILAB, set up in collaboration with AIST, Japan has the following equipment: Confocal microscope, Imaging & plate Reader, *In vivo* Imager, Stereomicroscope, and Fluorescence microscopes (2).

Cell Biology: A high-end FACS analyzer is available for cell counting and biomarker detection. In addition, a Blood Cell Analyzer is available for use. A BSL-2 facility for cell culture work is fully equipped with microscopes, CO_2 incubators, cell culture hoods, and cell storage facility.

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 & Metal jet) with optics, detectors and cryostreams.

Nuclear Magnetic Resonance (NMR) Spectrometer: 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.

Genomics Facility: A Quant Studio 6 and 7500 Fast RT-PCR from Invitrogen is available for quantitative analysis of gene and biomarker discovery.

Proteomics and Molecular Interaction platform: Mass Spectrometers, HPLC, Nano LC spotter, 2-D gel electrophoresis system and a protein sequencer are part of this facility. Instruments for probing molecular interactions include Surface Plasmon Resonance Unit, Isothermal Titration Calorimetry Unit, Differential Scanning Calorimetry system, Multipurpose Plate Readers, Dynamic Light Scattering Instrument, UV spectrophotometer, IR spectrophotometer, Fluorimeter and CD Spectro-polarimeter.

Also, equipment such as a laser scanner for biomolecular imaging, gel documentation units, RT-PCR machine and nanodrop spectrophotometer are also available. 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 also 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. For the students of the

Integrated MSc-PhD course, spacious and well-equipped teaching laboratory has been operationalized.

The Centre has facilities for classroom teaching, laboratory meetings, interactions, discussions, teaching and tutorials. The common facilities of the NCR Biotech Science cluster include auditorium complex, which has two seminar rooms (each with an occupancy of 150 persons) and one such room is available at all times for the Centre. The central auditorium is utilized for organizing and conducting institutional meetings, seminars, workshops and conclaves, besides holding adequate space for hold poster presentations.

Digital Initiatives

The Computing Facilities at RCB are quite modern with the wi-fi ready campus and high speed internet connectivity through the National Knowledge Network provisioned to all users. The Centre has been functioning in conformity with the guidelines of the Government of India with regard to IPv6 implementation and is an active participant in the Government initiatives of the "Digital India Campaign". Biometric Attendance facility has been enabled and Access Control Machines have been installed for authorized access to the high-end laboratory facilities in the campus. The Centre started procurement through "GeM-Government eMarket Place" portal and successfully placed several orders in the last year. Recently, a Smart Classroom Facility with Digital Podium and an interactive projector was operationalized. Also, infrastructure for Remote Classroom Facility was put in place, to enable faculty located at distant locations to teach students present in RCB.

A very competent and experienced IT service support team has been put in place and the Centre has a highly attractive, user-friendly and dynamic web-site with online admissions and recruitment. In the near future, the academic and administrative activities in RCB will be conducted through a dedicated digital Enterprise Resource Planning platform.

RCB's e-Library

The library & e-library facility have 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 available to all the users of the Centre. This facility shall be enhanced in near future with state-of-the-art Information & Communication Technology (ICT) labs. The Centre has established a library with modern amenities to enable students to acquire basic knowledge in different areas of biotechnology and conduct advanced research in specialized fields. Institutional repository has been created and maintained at RCB and centralized DST-DBT institutional repositories (Science Central). Library conducts orientation and user awareness program for new members in facilitating access to e-journals, e-books, plagiarism detection tools, bibliography creating tools, digital library, etc. The functioning of library is being automated through an open source library management system. RCB members can access the online resources around the clock through their electronics devices (Desktops, laptops, mobiles, etc.) and also using common desktops placed in the library, class rooms, labs and other facilities.

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.

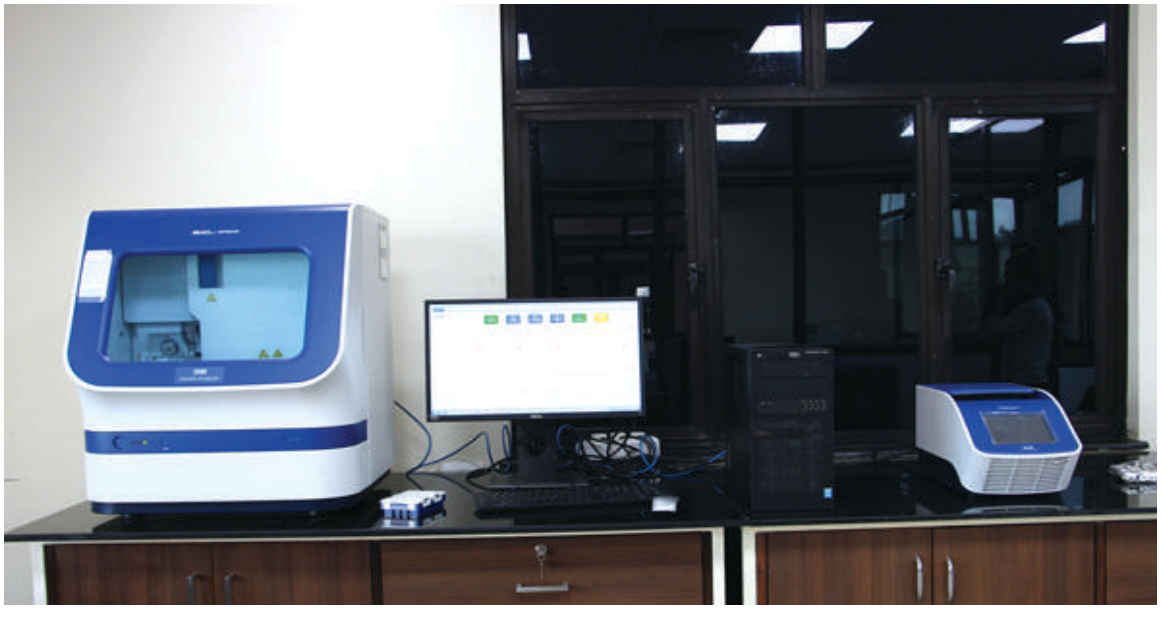
ATPC is in the process of creating six different technology platform for enabling research among the academic and industrial organizations. Out of six technologies, five technology platforms are operational.

I. **Protein Production, purification and interaction technologies**: This technology platform has two bioreactors of 7 L and 14 L having capability to run microbial and eukaryotic cell culture for protein production. The downstream workflows for purification of protein are fully operational with two Acta FPLC having different purification workflows. Interaction technology platform has been set up with Biolayer inferometry (BLI), Nano Temper MicroScale Thermophoresis (MST) and Multiangle light scattering technology (MALS). Each of these advanced technologies are used for label free interaction and molecular homogeneity of the proteins. All the above technologies are installed at ATPC and are in use on cost sharing basis.

II. **Mass spectrometry Facility:** Mass spectrometry facility of ATPC is equipped with one High throughput 5800 Plus MALDI TOF-TOF system, Nano LC with compatible plate spotter and High Flow HPLC for fractionation and separation of TMT /iTRAQ/SILAC labelled peptide for deeper coverage of whole proteome and PTM analysis. ATPC has recently acquired one High resolution ESI Q TOF (5600 Plus TOF –TOF) system for label free quantitation and biomarker discovery. Installation of this system is in process.

III. **Flow Cytometry Facility:** Flow cytometry facility is equipped with FACS analyzers (BD FACS verse, Beckman Coulter Galios and BD acquri C6 flow cytometer) and BD influx cell sorter. This facility is supported by several additional equipment to support the cell cultures of sorted cells that include CO_2 incubators, biosafety cabinet, centrifuges and inverted fluorescence microscope. Users from different institutes are using this facility for their experiments.

IV. **Genomics Facility:** Genomics facility of ATPC consist of eight capillary Sanger DNA sequencer and Droplet digital PCR. Both the instruments have been installed and this facility is providing support for the sequencing of DNA to the cluster institutes.



V. **Optical microscopy facility:** The high end optical microscopy facility of the ATPC will be catering to the need of diverse range of scientist in the academia and biotechnology start-ups. This facility has Confocal based high content imaging system (Molecular devices - Image express micro) with robotic for liquid handling and integrated environmental chamber and CO_2 incubators for *in vitro* screening of small molecule/si-RNA library or any high throughput screening project for academia and industry. The high throughput imaging facility is supplemented by Zeiss confocal microscope (LSM 880) and Zeiss super resolution microscope (structures illumination based Elyra PS 1 BD PALM microscope). The entire instrument in this facility is getting installed and staff is getting trained to operate these equipment.

VI. **Electron Microscopy Facility:** Electron Micoscopy facility of the ATPC is unique and one of its kind in North India, which will house the 200 kVA Direct electron detector CryoTEM (Jeol) and one Serial block facing FE-SEM (FEI). Along with these two electron microscopes, ATPC will soon have one more 120 kVA TEM for non-cryo application of biological sample. These electron microscopes have been ordered and expected to be installed in the ATPC in next two to three months.

At present, five facilities are operational in ATPC and these include the Protein Purification and Molecular Interactions Facility, Flow Cytometry facility, Mass Spectrometry Facility, Optical Microscopy and Genomics facility. Facilities 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.

The BSC BioNEST Bio-Incubator (BBB)

The Bio-Incubator at the NCR Biotech Science Cluster (BSC) is funded by BIRAC under the BioNEST (Bioincubators Nurturing Entrepreneurship for Scaling Technologies) scheme. The BSC BioNEST Bio-Incubator (BBB) is managed and operated by Regional Centre for Biotechnology. With the objective of fostering Indian biotech innovation and entrepreneurship, it aims to help start-ups, nurture their innovative ideas and develop globally competitive products and technologies. The BioIncubator aims to help emerging companies gain access to mentors, training programmes, shared space, professional assistance, capital and other services that will move them on to the fast track to success.

The BBB will benefit a wide range of stakeholders in BSC and beyond. Key stakeholders include incubatee companies/entrepreneurs and their employees, the community at large, and educational institutions. Companies/individuals that will incubate at BBB will enjoy dramatically improved success

rates as they would have an easy start in having affordable office space, access to shared equipment, meeting facilities and on-site business and technical assistance. BBB will also facilitate networking sessions on company formation, IP consultancy, potential source of capital in addition to providing access to domain experts in the field of science and technology.

The BBB is chiefly focused on life sciences, biopharma, bio-med-tech and allied areas for incubation. The 35,000 square feet covered area consists of labs of different denominations for incubation. It offers

shared lab benches as well as independent cubicles to incubatees to choose as per their requirement. Two common instrument labs have been set up that house basic to high-end equipment to support the start-ups in addition to instrument workshop. The incubator also provides adequate space for conducting meetings, videoconferencing, and seminars. Incubatees will also have access to the Advance Technology Platform Centre (ATPC), which houses state-of-the-art instrumentation facility and to the pool of expert scientists in various fields at RCB and other institutes in the Biotech Science Cluster. Selection process for incubation and facility usage charges have been approved by the BBB Advisory Committee. The facility is expected to open for incubation by 3Q, Fy2018.

Technology Advancement Unit (TAU)

The Indo-Swiss Collaboration in Biotechnology (ISCB) is a bilateral research and development programme, jointly funded and steered by the Swiss Agency for Development & Cooperation (SDC) and Department of Biotechnology (DBT). The mandate of ISCB is to develop products and biotechnological processes, which can be used or applied for the benefit of the rural communities in India and to build capacities of Indian institutions and promote partnerships between Swiss and Indian institutes as well as private companies of the two countries. SDC has established a Project Monitoring Unit (PMU) in Switzerland to facilitate and monitor the projects carried out under ISCB. 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) until October 2019. DBT had also sanctioned the project "Establishment of Technology Advancement Unit (TAU)" in 2014 for 5 years at the Regional Centre for Biotechnology as an adjunct to the Project Management Unit (PMU) of SDC with following objectives:

1. Create a conducive environment for product development and technology advancement & transfer as facilitator, advisor, and supporter for the project partners of the ISCB programme

through the facilitation of access to new skills and expertise.

2. Implementation of all activities of ISCB in extension and adoption by farmers.
3. Coordination of networking among stakeholder groups along the whole value chain.
4. Lead in monitoring at the project/network level along the whole value chain.
5. Build-up public awareness of ISCB activities, and in general in life sciences and biotechnology.
6. Coordination and organization of capacity building of ISCB partners.
7. Provide support service to DBT in dealing with ISCB project funds.
8. Provide technical/ scientific support to PMU for planning, reporting, and monitoring of ISCB projects at programme level.

Progress of work during the year 2017-18

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

(B) Ongoing ISCB projects

(A) ***Technology Transfer and Product Development (Technologies developed in Phase III)***

Projects	ISCB network partners	Product development partners	Mode of collaboration
Chickpeas resistant against pest insect pod borer (Cry1Ac)	Indian partner: ➢ AAU (Jorhat) Swiss partner: ➢ University of Basel	Indian partner (private): ➢ Sungro (Delhi) Indian partners (public): ➢ Punjab Agricultural University (Ludhiana) ➢ Indian Institute of Pulse Research (Kanpur)	License (With private partner) Material transfer agreement (With public partner)
Marker-free transgenic technology for resistance to pod borer using Bt (Cry2Aa)	Indian partner: ➢ AAU (Jorhat) Swiss partner: University of Basel	Indian partner (private): ➢ Sungro (Delhi) Indian partners (public): ➢ Punjab Agricultural University (Ludhiana)	License (With private partner) Material transfer agreement (With public partner)
Chickpeas resistant against pest insect aphid (ASAL)	Indian partner: ➢ Bose Institute Swiss partner: ➢ University of Basel	Private Indian partners ➢ Sungro (Delhi) ➢ Bioseed (Hyderabad)	License (With private partner)
Biofertilizer to improve yield and quality of wheat and rice	Indian partners: ➢ IIT (Delhi) ➢ GB PUAT (Pantnagar) ➢ TERI, (Delhi) Swiss partners: ➢ FiBL (Frick) ➢ University of Basel ➢ University of Neuchâte	Indian partner (private) : NFCL (Hyderabad)	License (With private partner)

The details of the four ongoing network projects and their progress & technology advancement are as follows:

1. **Project**: "Biofertilization and Bioirrigation for sustainable mixed cropping of pigeon pea and finger millet".

   Partners: 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) Laenggasse

   Expected Outcomes: Development of sustainable pigeon pea & finger millet intercropping systems based on the selection of responsive cultivars and the validated process of biofertilizers & bioirrigations.

   Current status: Field trials already on-going along with Socio-economic partners (eco-enterprise).

2. **Project**: "Indo Swiss Cassava Network".

   Partners: Central Tuber Crops Research Institute, Thiruvananthapuram; Tamil Nadu Agriculture University, Coimbatore, University of Basel.

   Expected Outcomes: Development of virus and white fly resistance in Cassava varieties.

   Current status: Facilitation in biological material transfers, building of seed distribution/ replacement systems in cassava in a multiparty approach and disseminate technology and ensure extension through AICRPTC.

3. **Project**: "Ragi Network".

   Partners: University of Agricultural Sciences, Bangalore, National Academy of Agricultural Research Management (NAARM), Hyderabad, ETH Zurich, University of Zurich (UZH), Functional Genomics Centre, Zurich (FGCZ)

   Expected Outcomes: Genetic enhancement, nutritionally improved and climate resilient Finger millet (Ragi).

   Current status: TAU facilitated in biological material transfers, GWAS studies, Validation of QTLs for selected shortlisted traits, promoted advancing of few selected lines from already characterized germplasm through All India Coordinated Millet Improvement Program.

4. **Project**: "Pigeon Pea Network".

   Partners: 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) Laenggasse

   Expected Outcomes: Improved Pigeon pea for plant type, pod borer resistance, and moisture stress tolerance.

   Current status: TAU facilitated in protecting Intellectual property of developed Micro-chip and identified a large number of lines in internal evaluation. Advancement of shortlisted entries will enter into AICRP trials.

Biosafety Support Unit (BSU)

Indian Biosafety Regulations implemented under the Environment (Protection) Act, 1986 are dynamic and complementing with emerging technologies used in research, product development, and progress in science of risk assessment. Department of Biotechnology, Government of India has undertaken several reforms in biosafety regulatory system including the "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 industry for generating biosafety data to address the challenges raised by the emerging areas of Biotechnology, and

(c) providing scientific information on emerging biosafety issues.

The BSU has undertaken the following major activities during the year 2017-18.

1. RCGM/GEAC Related Activities

BSU evaluated applications submitted to RCGM for consideration in RCGM meetings (153rd to 164th Meetings) during the year 2017-18 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 2017-18. During 2017-18, BSU prepared 34 RARMP documents in agriculture and analysed 48 Import/Export/Transfer/Receive applications, 24 C3 PCT applications, 22 C5 Preclinical Toxicology reports and 26 revised applications of C3 and C5. The support of BSU has facilitated RCGM in bringing down the approval time of 180 days to 90 days and efforts are on to reduce it further.

2. Launch of Indian Biosafety Knowledge Portal

BSU scientists carried out dummy cliental verification of Indian Biosafety Knowledge Portal (IBKP) and identified required changes to be made as per the current requirements by the regulators and the agency has been asked to rectify the issues accordingly. Further, BSU has updated and revised application proforma for online submission in the Indian Biosafety Knowledge Portal.

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. The "Regulations and Guidelines for Recombinant DNA Research and Bio-containment 2017" has now been released by Secretary, DBT. The revision of "Guidelines and Handbook for IBSCs 2011" is under progress and is likely to be completed shortly.

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 document 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 food wherein best international practices was taken into account. Further, a document covering the global picture on processing aids in food derived from GE organism and a draft proposal for Indian regulations on GM food have also been prepared.

5. Other activities

i. BSU has prepared (a) RARMP of DDGS (Distiller's Dried grains with solubles) Application for import of DDGS (b) RARMP on genetically modified Mosquito, (c) Evaluation report on *Wolbachia* infected *Aedes aegptii* and *Anopheles* mosquito, (d) GE silk worm documents, (e) draft AFES for the dossier on the application for insect resistance for Dow Agrosciences.

ii. Department of Agriculture & Cooperation has issued a notification based on the report of BSU specifying minimum level of BT toxin (Cry1Ac and Cry2Ab) for cotton seed lots for labelling of BT genes in cotton.

iii. BSU has facilitated RCGM for implementing simplified procedure for import/ export of GMOs and presently assisting RCGM for simplifying event selection under small- scale confined area but within the institute/ company owned land with the approval of IBSC under intimation to RCGM and relaxation of requirement of NOC from State Government, as approved by GEAC in its 134th meeting held on 21-03-2018.

iv. BSU has extended support to DBT for assessing extent and veracity of unapproved cultivation of HT cotton. BSU facilitated the Field inspection and scientific evaluation committee (FISEC) by providing scientific documents, scientific evaluation of samples, on-the spot inspection of fields and preparing the draft report.

v. BSU has also been involved in various monitoring teams to ensure compliance during confined field trials/ facility evaluation etc.

vi. Support to RCGM/GEAC for drafting affidavits/ replies for various Supreme Court cases and assessment of the report of Centre for Science and Environment on GM food. 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, stability studies data requirements, canine research center globally, bivalent measles vectored HPV vaccines, Bovine Paratuberculosis/ Johne`s Disease, Frozen Aviary project, Pertuzumab, and the Future Belongs to Biologics.

vii. BSU collected data about all the functional and active IBSCs in India. The database is being maintained at BSU/RCGM. A number of IBSCs have been reconstituted with new DBT nominees.

viii. BSU facilitated RCGM secretariat to collect data from all over India through respective IBSCs for BSL3 and BSL4 facilities. There are 28 functional BSL3 and above facilities across India.

6. Scientific meetings attended/ organized

I. Under Training and Capacity Building, BSU Scientists have attended India-US strategic dialogue on Biosecurity at Washington DC (November 6-7, 2017), New Delhi (February 8-9, 2017 and February 8-9, 2018); on Synthetic Biology SB 7.0 at National University of Singapore (June 13-16, 2017) and 14th ISBGMO at Mexico, June 4-8, 2017.

II. A one-day Workshop was organized by BSU in association with TERI for the African Nationals as part of the Biotechnology and biosafety study tour to India on 'Food/Feed & Environmental Risk Assessment: Indian Perspective'. The workshop was attended by 12 delegates from African countries like Nigeria, Senegal, National Biosafety Authority (NBA), Zambia, African Biosafety Network of Expertise (ABNE), Burkina Faso and a Participant from Michigan State University, USA. The objective of the workshop was to provide detailed information on the Regulatory Framework for Safety Assessment of GM crops in India and Indian perspective on Assessment of Food, Feed and Environmental Safety of GM crops. The outcome of the workshop was deeper understanding of the biosafety regulatory framework and process followed for biosafety assessment and understanding of the common challenges faced in the arena of GM crops and reforms brought by regulatory agencies of both the geographies i.e Indian subcontinent and African subcontinent.

Presentations made by BSU

Scientists of BSU have presented their work in various international (ISBGMO 2017) and regional (SABC 2017) forums through talks and posters such as:

a) Talk on Biosafety assessment for environmental release of genetically engineered (GE) Algae: An Indian Perspective

b) Poster - Modelling of Risk Assessment of Above (AGE) & Below (BGE) Ground GE Plant Developed Through Grafting

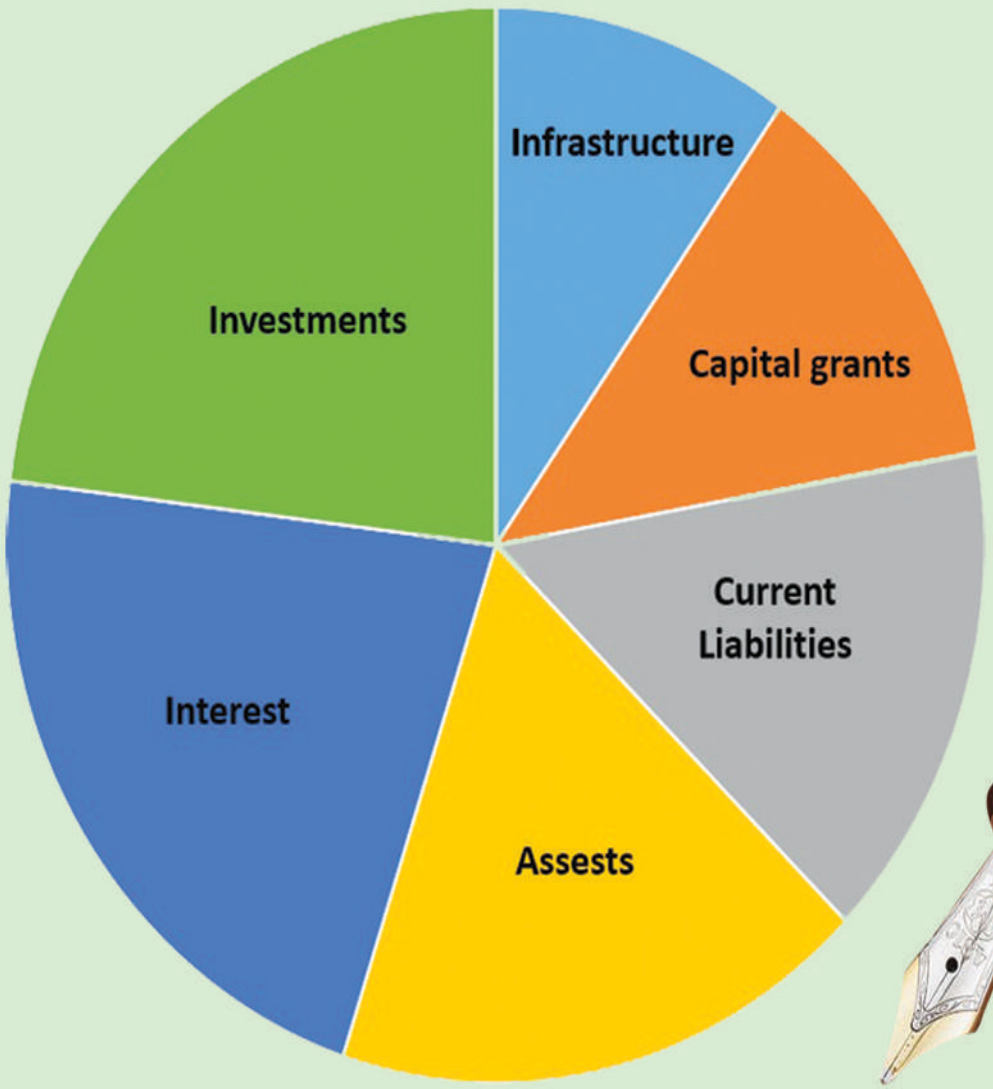
c) Poster - Assessment of impact of Genetically Engineered (GE) mustard (*Brassica juncea* l.) on honey bees (a draft manuscript has been prepared on this aspect).

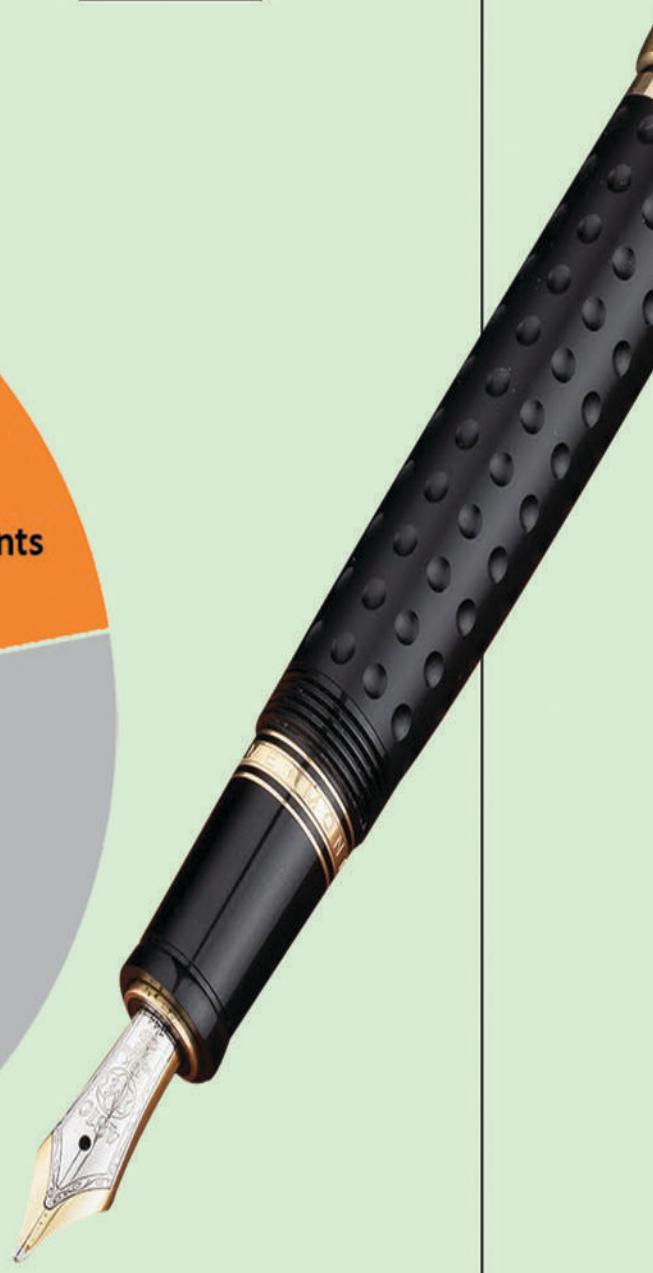
d) Poster - Meta-analysis of data on the expression of Cry proteins and field performance of Bt cotton hybrids approved in India (a draft manuscript has been prepared on this aspect)

e) Poster - Assessment of impact of gene flow on biodiversity: Experience with GE mustard

Balance Sheet — **Regional Centre for Biotechn**

Liabilities	Regional Centre for Biotechnology as at 31-Mar-2018		Assets
Capital Account		15,89,46,572.00	Fixed Assets
Loans (Liability)			Investments
Current Liabilities		2,96,24,24,894.26	Current Asse
Excess of income over expenditure		4,15,012.00	
Opening Balance	*4,15,012.00*		
Current Period			



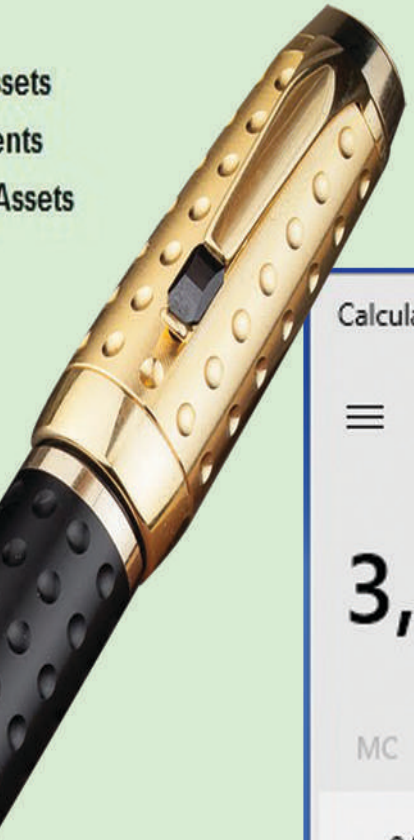


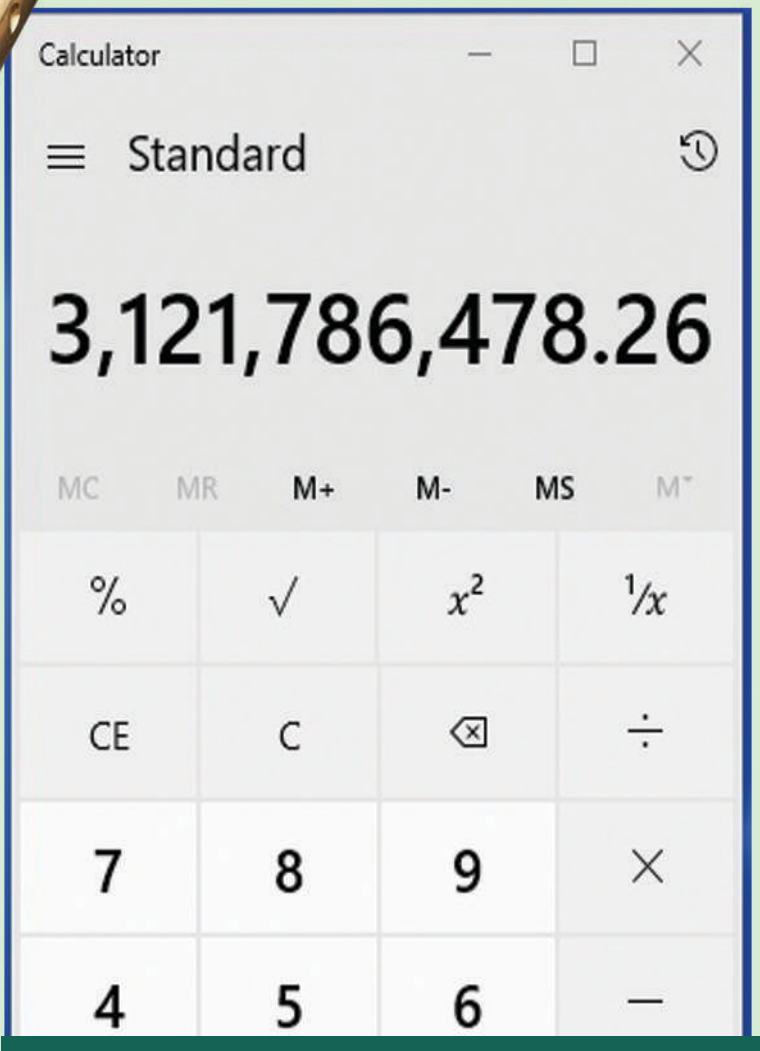
Total	3,12,17,86,478.26	Total

Regional Centre for
Biotechnology
as at 31-Mar-2018

t s

sets	2,13,01,84,081.88
nts	26,09,97,100.00
Assets	73,06,05,296.38





FINANCIAL STATEMENTS

	3,12,17,86,478.26

REGIONAL CENTRE FOR BIOTECHNOLOGY
BALANCE SHEET AS AT 31ST MARCH, 2018

Amount (In Rs.)

CORPUS / CAPITAL FUND AND LIABILITIES	Schedule		Current Year		Previous Year
CAPITAL GRANTS FOR INFRASTRUCTURE	1	-	1589,46,572	-	1666,00,236
RESERVES AND SURPLUS	2	-	4,15,012	-	4,15,012
CURRENT LIABILITIES AND PROVISIONS	7(A)	-	6113,93,319	-	566,11,128
BIOTECH SCIENCE CLUSTER (BSC)	7(B)	-	22889,71,991		21968,73,773
TOTAL			**30597,26,894**		**24205,00,149**
ASSETS					
FIXED ASSETS	8	-	1432,10,252	-	1588,23,292
INVESTMENTS - OTHERS	10 & 11 (B)	-	2575,97,100	-	-
CURRENT ASSETS,LOANS ADVANCES ETC.	10 & 11(A+C)	-	5548,55,187	-	2677,84,714
BIOTECH SCIENCE CLUSTER (BSC)	10 & 11(D)		21040,64,355	-	19938,92,143
a. Capital Work in progress	8	19869,73,829		19519,46,843	
b. Advance to BSC construction.	10 & 11(D ii & iii)	1062,43,350		311,38,229	
c. Funds in short term deposits	10 & 11 (D i)	34,00,000		34,00,000	
d. Accrued interest & TDS	10 & 11(iv+v)	74,47,176		74,07,071	
TOTAL			**30597,26,894**		**24205,00,149**
SIGNIFICANT ACCOUNTING POLICIES	24		Enclosed Separately		
NOTES TO ACCOUNTS, CONTINGENT LIABILITIES	25		Enclosed Separately		

BIJU MATHEW
SENIOR MANAGER (A&F)

बिजू मैथ्यु / Biju Mathew
वरिष्ठ प्रबन्धक (प्र० एवं वित्त) / Senior Manager (A&F)
क्षेत्रीय जैवप्रौद्योगिकी केन्द्र / Regional Centre For Biotechnology
(जैवप्रौद्योगिकी विभाग, भारत सरकार द्वारा स्थापित)
(Estd. by the Dept. of Biotechnology, Govt. of India)
भारत सरकार यूनेस्को के तत्वावधान में
Under the auspices of UNESCO
एन. सी. आर. बायोटेक साईंस क्लस्टर / NCR, Biotech Science Cluster
तृतीय मील पत्थर, फरीदाबाद- गुड़गांव एक्सप्रेस वे
3rd Mile Stone, Faridabad- Gurgaon Expressway
फरीदाबाद-121001, हरियाणा / Faridabad-121001, Haryana

PLACE: Faridabad
Date 10/8/2018

SUDHANSHU VRATI
EXECUTIVE DIRECTOR

प्रो. सुधांशु व्रती / Prof. Sudhanshu Vrati
कार्यपालक निदेशक / Executive Director
क्षेत्रीय जैवप्रौद्योगिकी केन्द्र / Regional Centre for Biotechnology
फरीदाबाद - 121 001 (हरियाणा), भारत/Faridabad - 121 001 (Haryana), India

REGIONAL CENTRE FOR BIOTECHNOLOGY

SCHEDULES FORMING PART OF INCOME & EXPENDITURE ACCOUNT FOR YEAR ENDED 31st MARCH, 2018

Amount (In Rs.)

SCHEDULE 22 - Status of Contribution from Constituents of the NCR Biotech Science Cluster& Expenditure	Opening Balance on 1.4.2017	Additions during the year 2017-18	Closing balance as on 31.3.2018
Contribution and interest			
1. Translational Health Science & Technology Institute	10116,38,536	-	10116,38,536
2. National Institute of Immunology	1879,02,000	-	1879,02,000
3. Regional Centre for Biotechnology	6500,65,000	-	6500,65,000
4. BIRAC Bio-Incubator	2070,15,848	279,00,000	2349,15,848
5. Advance Technology Platform Centre	577,22,000	398,84,000	976,06,000
6. Interest on investment of BSC Funds	722,16,030	4,19,413	726,35,443
Total Contribution from constituents	**21865,59,414**	**682,03,413**	**22547,62,827**
Advances, Expenditure and outflow			
1. Capital Work in progress	19519,46,843	350,26,986	19869,73,829
2. Advance to BSC construction.	311,38,229	751,05,121	1062,43,350
3. Margin money against security in MCF	34,00,000	-	34,00,000
4. Accrued interest & TDS	74,07,071	40,105	74,47,176
Total funds utilized	**19938,92,143**	**1101,72,212**	**21040,64,355**
Balance of NCR funds with RCB	**1926,67,271**		**1506,98,472**

BIJU MATHEW
Sr. Manager (A&F)

PLACE: Faridabad
Dated : 10/8/2018

बिजू मैथ्यु / Biju Mathew
वरिष्ठ प्रबन्धक (प्र० एवं वित्त) / Senior Manager (A&F)
क्षेत्रीय जैवप्रौद्योगिकी केन्द्र / Regional Centre For Biotechnology
(जैवप्रौद्योगिकी विभाग, भारत सरकार द्वारा स्थापित)
(Estd. by the Dept. of Biotechnology, Govt. of India)
भारत सरकार यूनेस्को के तत्वावधान में
Under the auspices of UNESCO
एन. सी. आर. बायोटेक साईंस क्लस्टर / NCR, Biotech Science Cluster
तृतीय मील पत्थर, फरीदाबाद- गुड़गांव एक्सप्रेसवे
3rd Mile Stone, Faridabad- Gurgaon Expressway
फरीदाबाद-121001, हरियाणा / Faridabad-121001, Haryana

SUDHANSHU VRATI
(Executive Director)

प्रो. सुधांशु व्रती / Prof. Sudhanshu Vrati
कार्यपालक निदेशक / Executive Director
क्षेत्रीय जैवप्रौद्योगिकी केन्द्र / Regional Centre for Biotechnology
फरीदाबाद - 121 001 (हरियाणा), भारत/Faridabad - 121 001 (Haryana), India

Regional Centre for Biotechnology

Schedule 24 : Accounting Policies and Notes Forming Parts of the Balance Sheet and Income & Expenditure Account for the Year Ended at 31st March, 2018

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

2. Since the RCB bill has been passed and notified on 1.3.2017 and thereafter the Statutes, Ordinances and regulations approved during September 2017, the liabilities on account of Gratuity & leave encashment of the Centre has been incorporated in the accounts for FY 2017-18 in accordance with the approved service conditions of the RCB, based on actuarial valuation.

3. (a) Recurring Grants have been recognised in the Income & Expenditure account and non-recurring Grants have been shown as part of capital.

   (b) Grants for core funds relatable to depreciable fixed assets are treated as deferred income and recognised in the Income and Expenditure Account on a systematic and rational basis over the useful life of such assets i.e. such grants are allocated to income over the periods and in the proportions in which depreciation in charged (As per Accounting Standard 12). During the year income recognised in respect of such Grants amounts to Rs. 576,53,664.00

4. (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. In respect of additions to/deductions from fixed assets during the year, depreciation is considered on pro-rata basis

5. (a) Fixed assets have been created with core grants received from the Department of Biotechnology. No equipment procured out of project funds have yet been capitalized.

   (b) Fixed Assets are stated at cost acquisition inclusive of inward freight, duties and taxes and incidental and direct expenses related to acquisition.

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

7. Further all entries relating to purchase of consumables /equipments or other fixed assets in accounts are being passed only at the time of submission of

satisfactory inspection/installation report irrespective of the date of actual receipt of the supplies / equipments.

8. Transactions denominated in foreign currency are accounted at the exchange rate prevailing at the date of transaction.

9. 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.

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

11. Expenses and Overheads incidental to construction of building of institute as well as other buildings in the NCR BSC, 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 final accounts by the PMC. The project is being operated with an agreement which stipulates operation of an Escrow Account by NCR Biotech Science Cluster. The authorized signatories are Engineers India Ltd. (Project Management Consultant)

12. The Institute has received contribution of Rs.2254,47,62,827.00 (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 1.4.2017	Received during 2017-18	Total receipts on 31.3.2018
1	THSTI	10116.39		10116.39
2.	NII	1879.02	-	1879.02
3.	RCB	6500.65	-	6500.65
4.	Bio-Incubator	2070.16	279.00	2349.16
5.	ATPC	577.22	398.84	976.06
6.	Interest on investment of BSC funds	722.16	4.20	726.35
	Total	**21865.59**	**682.04**	**22547.63**

and the total expenditure incurred as on 31st March 2018 against such contribution is amounted to Rs. 21040,64,355.00. 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.

13. 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.

Schedule 25 : Contingent Liabilities

1. Purchase orders for consumables worth Rs.81,68,697.00 ordered during 2017-18 are outstanding as on 31.3.2018 which have not be recognized in the books of accounts.

2. Purchase orders for Equipment worth Rs.66,06,581.00 ordered during 2017-18 are outstanding as on 31.3.2018 which have not been recognized in the books of accounts.

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

बिजू मैथ्यु / Biju Mathew
वरिष्ठ प्रबंधक (प्र० एवं वित्त) / Senior Manager (A&F)
क्षेत्रीय जैवप्रौद्योगिकी केन्द्र / Regional Centre For Biotechnology
(जैवप्रौद्योगिकी विभाग, भारत सरकार द्वारा स्थापित)
(Estd. by the Dept. of Biotechnology, Govt. of India)
भारत सरकार यूनेस्को के तत्वावधान में
Under the auspices of UNESCO
बायोटेक साईंस क्लस्टर / NCR Biotech Science Cluster
तृतीय मील पत्थर, फरीदाबाद-गुड़गांव एक्सप्रेसवे
3rd Mile Stone, Faridabad-Gurgaon Expressway
फरीदाबाद-121001, हरियाणा / Faridabad-121001, Haryana

Place: Faridabad
Date:10/8/2018

(Dr. Sudhanshu Vrati)
Executive Director

प्रो. सुधांशु व्रती / Prof. Sudhanshu Vrati
कार्यपालक निदेशक / Executive Director
क्षेत्रीय जैवप्रौद्योगिकी केन्द्र / Regional Centre for Biotechnology
फरीदाबाद - 121 001 (हरियाणा), भारत/Faridabad - 121 001 (Haryana), India

Separate Audit Report of Comptroller and Auditor General of India on the accounts of Regional Centre for Biotechnology, Faridabad for the year ended 31 March 2018

We have audited the attached Balance Sheet of Regional Centre for Biotechnology (RCB), Faridabad at 31 March 2018 and the Income and Expenditure Account/ Receipts and Payments Account for the year ended on that date under Section 19(2) of the Comptroller and Auditor General's (Duties, Powers and Conditions of Service) Act, 1971 read with section 32 (1) of RCB Act, 2016. These financial statements are the responsibility of the RCB's management. Our responsibility is to express an opinion on these financial statements based on our audit.

2. This Separate Audit Report contains the comments of the Comptroller and Auditor General of India on the accounting treatment only with regard to classification, conformity with the best accounting practices, accounting standards and disclosure norms, etc. Audit observations on financial transactions with regard to compliance with the Law, Rules & Regulations (Propriety and Regularity) and efficiency-cum-performance aspects, etc., if any, are reported through Inspection Reports/ Comptroller and Auditor General's Audit Reports separately.

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

4. Based on our audit, we report that –

(i) We have obtained all the information and explanations except those stated in the report, which to the best of our knowledge and belief were necessary for the purpose of our audit;

(ii) The Balance Sheet, Income and Expenditure Account and Receipts and Payments Account dealt with by this report have been drawn up in the format approved by the Government of India;

(iii) In our opinion, proper books of accounts and other relevant records have been maintained by RCB, except those stated in this audit report.

(iv) We further report that :

(A) BALANCE SHEET

1. **Corpus Fund- (Schedule-1)-Rs. 15.89 crore**

RCB included total Capital Grant of Rs. 500.00 lakh under the Capital/ Corpus fund whereas the amount of grant actually capitalized was Rs. 421.18 lakh which was to be shown under the corpus fund as additions during the year. Thus, there was difference of Rs. 78.82 lakh and this difference was to be shown under the Current Liabilities.

This has resulted in overstatement of Corpus Fund by Rs 78.82 lakh and understatement of the Current Liabilities against Government Grant to the same extent.

(B) **Income & Expenditure Account**

1 **Income: Interest on Fixed Deposits/Saving Account- (Schedule 18)-Rs. 45.07 lakh**

(a) Interest amounting to Rs 45.07 lakh earned on savings out of core grant were taken as its 'Income' in Income and Expenditure Accounts. This was to be reported/ refunded to DBT.

Thus, RCB overstated its Income and understated Current Liabilities towards 'Unspent grant refunded to Govt.' each by Rs 45.07 lakh.

2. **Expenditure:**

2.1 **Other Administrative Expenses:**

(Schedule 21: Rs. 1086.99 lakh)

Above included expenditure of Rs.31.70 lakh pertaining to previous years which should have booked under Prior Period Expenditure. This has resulted into overstatement of Other Administrative Expenses and understatement of Prior Period Expenditure by Rs. 31.70 lakh each.

(C): GRANT-IN-AID

RCB received total grant of Rs 20.65 crore from DBT during 2017-18, besides having carried forward unspent grant of Rs 1.83 crore for the year 2016-17. This apart interest income of Rs. 0.45 crore and revenue from internal resources of Rs. 0.69 crore was also received. Thus, RCB took the entire receipt of Rs. 23.62 crore as available for expenditure. RCB utilized a sum of Rs 21.21 crore and Rs.2.38 crore (prior period) leaving a balance of Rs 0.03 Crore was shown as unutilized grant as on 31 March 2018.

(D) General :

(a) RCB had shown Bio-tech Science Cluster (BSC) fund amounting to Rs. 22889.72 lakh in their own Accounts whereas this has been the amount of pooled funds of five cluster entities e.g. THSTI, NII and Bio-Incubator etc. including only Rs. 6500.65 lakh for RCB . The funds other than RCB needs to be shown outside this regular Account of RCB.

(b) The buildings and roads costing Rs. 6500.65 lakh though completed and handed over for use in March 2015 has not yet been reflected under Assets. No depreciation has been charged on it.

(E) Management letter

Deficiencies which have not been included in the Audit Report have been brought to the notice of the Executive Director RCB through a management letter issued separately for remedial/corrective action.

(v) Subject to our observations in the preceding paragraphs, we report that the Balance Sheet, Income & Expenditure Account and Receipts & Payments Account dealt with by this report are in agreement with the books of accounts.

(vi) In our opinion and to the best of our information and according to the explanations given to us, the said financial statements read together with the Accounting Policies and Notes on Accounts, and subject to the significant matters stated above and other matters mentioned in Annexure to this Audit Report give a true and fair view in conformity with accounting principles generally accepted in India.

(a) In so far as it relates to the Balance Sheet, of the state-of-affairs of the RCB as of 31 March 2018; and

(b) In so far as it relates to Income and Expenditure Account of the neither surplus nor deficit for the year ended on that date.

For and on behalf of CAG of India

Dated :
Place : New Delhi

Manik Kumar
Director General of Audit
(Scientific Departments)

Annexure – I

(A) Adequacy of Internal Audit System

Internal Audit of the Regional Centre for Bio-technology (RCB) was required to be conducted by the internal audit wing of Principal Pay & Accounts Office of the Department of Science & Technology, New Delhi which was completed upto March 2017. A total number of 34 paras (one pertained to the period 2010-14 and 33 pertained to the period 2014-17) were outstanding till date (September 2018).

(B) System of Physical Verification of Fixed Assets

Register of Fixed Assets was not being maintained in RCB as per Rule 211(ii)(a) in Form GFR-22, it was further observed that physical verification of fixed Assets has never been conducted.

(C) System of Physical verification of inventory

The Physical verification of Consumable items and Materials has been carried out for the period upto 2013-14 and no discrepancy was reported. It was, however, observed that the physical verification report was not as per approved format as per GFRs. There was no facility for Central Store in RCB. The registers for stock entries were maintained but there was no central store facility in absence of which physical count of store items was not possible as the inventories were issued directly to the indenting sections.

(D) Regularity in payment of statutory dues:

RCB was generally regular in payment of Statutory Dues. An amount of Rs. 35.68 lakh was, however, due as duties and taxes to be paid as of 31st March 2018.

(E) Adequacy of Internal Control System

a) Internal control system of RCB was adequate; however, RCB did not have its Accounting Manual and standard guidelines/manual for purchase/ procurement.

b) Bank Reconciliation Statement

RCB furnished the bank reconciliation statement for the month of March 2018 which revealed that in Saving bank account cheques issued amounting to Rs. 269.31 lakh by RCB were not reflected in the bank and out of it 11 cheques amounting to Rs. 0.14 lakh became time barred and in Current Accounts cheques amounting to Rs. 27.92 lakh were not reflected in the bank out of

which 7 cheques received and deposited in banks amounting to Rs. 6.62 lakh and 36 cheques issued but not presented in the bank amounting to Rs. 4.26 lakh have become time barred.

c) Though the provisional Utilization Certificate(s) for the financial year 2017-18 in respect of the grants-in-aid received by RCB from DBT were sent to Ministry but it was not as per Form 12-A (under Rule 238-I) of GFRs 2017.

Director (Insp.)

PROFORMA

Separate Audit Report on the accounts of Regional Centre for Biotechnology, Faridabad for the year 2017-18

1.	Date of submission of the accounts to the Audit by the autonomous body	13.08.2018
2.	Where applicable reasons for returning the accounts for revision indicating why the accounts could not be certified with qualification	NA
3.	Date of submission of revised accounts to Audit where revision was considered essential	N.A.
4.	Dates on which audit was taken up and completed	11.09.2018 to 28.9.2018
5.	(a) Date of issue of SAR to Head Office (b) Date of issue of draft SAR to Autonomous Body for replies comments. (c) Date of issue of draft SAR to Head Office after incorporating reply of Autonomous Body	16.10.2018
	Date of receipt of replies/ comments from Autonomous Body (if received)	26.11.2018
6.	Date of issue of draft SAR including replies/ comments of Autonomous Body alongwith an Aide-memoire to CAG's office for approval	01.01.2019
7.	(a) Date of CAG's office letter communicating approved SAR. (b) Date of receipt of letter and approval at 8(a)	08.01.2019
8.	Date of issue of final Audit Report to Govt. of India/ State Govt./ CAG's office	
9.	Reasons for delay, if any at various stages	
10.	Dates of presentation of the previous Audit Reports before Parliament/ Legislature (Where the Audit Report for previous years have not been placed, years to which these pertain, may also be indicated)	

Year	Lok Sabha	Rajya Sabha
NA	NA	NA
NA	NA	NA

Manish Kumar

Director General of Audit (SD)

INSTITUTIONAL GOVERNANCE

Board of Governors (BOG)

Dr. Renu Swarup **(Chairperson)**
Secretary, Department of Biotechnology
Block-2, 7th Floor CGO Complex, Lodhi Road
New Delhi - 110 003

Prof. M. Radhakrishna Pillai **(Ex-officio Member)**
Director, Rajiv Gandhi Centre for Biotechnology
Poojappura, Thiruvananthapuram - 695 014
Kerala

Prof. Saumitra Das **(Ex-officio Member)**
Director, National Institute of Biomedical Genomics
Netaji Subhas Sanatorium and Tuberculosis Hospital, 2nd Floor
P.O.: N.S.S., Kalyani 741 251
West Bengal

Prof. Gagandeep Kang **(Ex-officio Member)**
Executive Director, Translational Health Science & Technology Institute
NCR Biotech Science Cluster
Faridabad - 121 001

Mr. Eric Falt **(Ex-officio Member)**
Director, UNESCO Delhi Office
UNESCO House, 1, San Martin Marg, Chanakyapuri
New Delhi - 110 021

Prof. Y. K. Gupta **(Permanent Invitee)**
Professor & Head, Department of Pharmacology
All India Institute of Medical Sciences
New Delhi - 110 029

Dr. Alka Sharma **(Special Invitee)**
Advisor & Coordinator for RCB
Scientist-G, Department of Biotechnology
Govt. of India, New Delhi

Dr. Nitin Jain **(Special Invitee)**
Scientist-E and Nodal Officer for RCB
Department of Biotechnology
Govt. of India, New Delhi

Prof. Sudhanshu Vrati **(Convenor)**
Executive Director
Regional Centre for Biotechnology
NCR Biotech Science Cluster
Faridabad - 121 001

Programme Advisory Committee (PAC)

Dr. Y. K. Gupta **(Chairperson)**
Former Professor & Head
Department of Pharmacology
All India Institute of Medical Sciences, New Delhi 110 029

Dr. Debashis Mitra **(Member)**
Director
Centre for DNA Fingerprinting and Diagnostics, Hyderabad 500 039

Dr. Alka Sharma **(Member)**
Advisor
Scientist-G, Department of Biotechnology, New Delhi 110 003

Dr. V. Vaidya **(Member)**
Professor
Department of Biological Sciences
Tata Institute of Fundamental Research, Mumbai 400 005

Dr. Rashna Bhandari **(Member)**
Staff Scientist
Centre for DNA Fingerprinting and Diagnostics, Hyderabad 500 039

Dr. Shrikumar Suryanarayan **(Member)**
Chairman, Sea6 Energy, Bengaluru 560 065

Dr. Paramjit Khurana **(Member)**
Professor & Head
Department of Plant Molecular Biology
University of Delhi South Campus
New Delhi 110 021

Prof. Rakesh Bhatnagar **(Member)**
Vice-Chancellor
Banaras Hindu University
Varanasi 221 005

Dr. Joel Sussman **(Member)**
Professor, Dept. of Structural Biology
The Weizmann Institute of Science, Israel

Prof. Angelo Azzi **(Member)**
Vascular Biology Laboratory
Tufts University, USA

Prof. R. Venkata Rao **(Member)**
Vice Chancellor
National Law School of India University, Bangalore 530 072

Dr. Nitin Jain **(Special Invitee)**
Scientist-E
Department of Biotechnology, Govt. of India, New Delhi

Prof. Sudhanshu Vrati **(Member-Secretary)**
Executive Director
Regional Centre for Biotechnology, Faridabad 121 001

Executive Committee (EC)

Prof. Sudhanshu Vrati **(Chairman, Ex-officio)**
Executive Director
Regional Centre for Biotechnology
Faridabad 121 001

Sh. Chandra Prakash Goyal **(Member, Ex-officio)**
Joint Secretary (Administration)
Department of Biotechnology
Govt. of India, New Delhi 110 003

Mr. Eric Falt **(Member, Ex-officio)**
Director & UNESCO representative to
Bhutan, India, Maldives and Sri Lanka
UNESCO Office, New Delhi 110 021

Dr. Alka Sharma **(Special Invitee)**
Advisor and Coordinator for RCB
Scientist-G, Department of Biotechnology
Govt. of India, New Delhi 110 003

Dr. Nitin Jain **(Member, Ex-officio)**
Scientist-E and Nodal Officer for RCB
Department of Biotechnology
Govt. of India, New Delhi

Dr. N. Saravana Kumar **(Member, Ex-officio)**
Joint Secretary (ICC)
Ministry of Human Resource Development
Govt. of India, New Delhi 110 066

Joint Secretary **(Member, Ex-officio)**
UNES Division
Ministry Of External Affairs
Govt. of India, New Delhi 110 001

Registrar **(Permanent Invitee)**
Regional Centre for Biotechnology
Faridabad 121 001

Finance Officer **(Permanent Invitee)**
Regional Centre for Biotechnology
Faridabad 121 001

Controller of Administration **(Member-Secretary, Ex-officio)**
Regional Centre for Biotechnology
Faridabad 121 001

Finance Committee (FC)

Prof. Sudhanshu Vrati **(Chairman, Ex-officio)**
Executive Director
Regional Centre for Biotechnology
Faridabad 121 001

Mr. B. Anand **(Member, Ex-officio)**
Additional Secretary & Financial Advisor
Department of Biotechnology
Govt. of India, New Delhi 110 003

Dr. Alka Sharma **(Special Invitee)**
Advisor & Coordinator for RCB
Scientist-G, Department of Biotechnology
Govt. of India, New Delhi 110 003

Dr. Nitin Jain **(Member Ex-officio)**
Scientist-E and Nodal Officer for RCB,
Department of Biotechnology
Govt. of India, New Delhi

Dr. Gagandeep Kang **(Member, Ex-officio)**
Executive Director, THSTI
Faridabad 121 001

Dr. Sandeep Chatterjee **(Nominated Member)**
Registrar, IIT-Delhi
New Delhi 110 016

Shri Pitambar Behera **(Nominated Member)**
Sr. Finance Officer
Indian Institute of Foreign Trade
New Delhi 110 016

Controller of Administration **(Member, Ex-officio)**
Regional Centre for Biotechnology
Faridabad 121 001

Shri Biju Mathew **(Member-Secretary, Ex-officio)**
Sr. Manager (Administration & Finance)
Regional Centre for Biotechnology
Faridabad 121 001

Board of Studies

Prof. Sudhanshu Vrati **(Chairman, Ex-officio)**
Executive Director
Regional Centre for Biotechnology
Faridabad 121 001

Dr. Prasenjit Guchchait **(Member)**
Professor
Regional Centre for Biotechnology
Faridabad 121 001

Dr. Deepak T. Nair **(Member)**
Associate Professor
Regional Centre for Biotechnology
Faridabad 121 001

Dr. C. V. Srikanth **(Member)**
Associate Professor
Regional Centre for Biotechnology
Faridabad 121 001

Dr. V. S. Bisaria **(Subject Expert)**
Professor
Dept. of Biochemical Engineering & Biotechnology
Indian Institute of Technology Delhi
New Delhi 110 016

Dr. Rajiv Bhat **(Subject Expert)**
Professor
School of Biotechnology
Jawaharlal Nehru University
New Delhi 110067

Dr. Goutam Ghosh **(Subject Expert)**
Senior Vice President
Vaccines and biological Research
Panacea Biotech Limited
New Delhi 110 044

Dr. Deepika Bhaskar **(Member-Secretary)**
Registrar
Regional Centre for Biotechnology
Faridabad 121 001

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
Dr. S. V. Eswaran

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

International Adjunct Faculty
Prof. Falguni Sen

Wellcome Trust-DBT IA Intermediate Fellow
Dr. Geetanjali Chawla
Dr. Geeta Ram
Dr. Pinky K. Sharma

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

DBT-BioCARe Awardee
Dr. Kanchan Bhardwaj

Young Investigators
Dr. Amit Kumar Yadav
Dr. Sunil Kumar Tripathi
Dr. Siddhi Gupta
Dr. Shivendra Pratap
Dr. Prabhakar
Dr. Yashika Walia Dhir
Dr. Raghavendra Aminedi

DST INSPIRE Faculty
Dr. Naini Burman

Research Fellows (PhD Scholars)

Harmeet Kaur
Amit Sharma
Sarita Chandan Sharma
Salman Ahmad Mustfa
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
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
Hridya Chandrasekar
Shreyasi Das
Manisha Kumari
Arunima Gupta
Krishnendu Goswami
Mritunjay Kasera
Zaid Kamal Madni
Chandan Kumar
Akriti Sharma
Shrimali Nishith
Maheshbhai
Sandhini Saha
Shraddha Kantilal Dahale
Animesh Kar
Anushree
Preksha Gaur
Sonalika Maurya
Priyanka Verma
Saibal Saha
Patterson Clement C
Harsh Kumar
Pergu Rajaiah
Smita Yadav
Jaya Saini
Amar Prajapati
Pankaj Kumar Sahoo
Arundhati Deb

Project Fellows

Research Associates/ Post-Doctoral Fellows
Dr. Teena Bhakuni
Dr. Madhurima Mitra
Dr. Amit Kumar Dey
Dr. Bhoj Kumar
Dr. Jewel Jameeta Noor
Dr. Amit Kumar Rajora

Senior Research Fellow
Abhishek Kumar Singh
Himani Sharma

Junior Research Fellows
Deepak Kumar Mishra
Mohammad Asad
Nishant Sharma
Divya Saxena
Parul Rani
Anil Kumar Singh
Rituparna Basak
Rajani Gupta
Hemant Nath Goswami
Sonali

Project Associates
Shrishti Sanghi

Lab Assistant
Varun Sharma

Field Operating Officer
Dr. Abha Jain

Management

Office of the Executive Director

Executive Director
Prof. Sudhanshu Vrati

Staff Officer to Executive Director
Dr. Nidhi Sharma

Technical Assistant
Mr. C. Ramesh

Administration, Finance, and Purchase

Senior Manager (A&F)
Mr. Biju Mathew

Administrative Officer
Mr. V.M.S. Gandhi

Section Officer
Mr. Rakesh Kumar Yadav
Mr. Sanjeev Kumar Rana
Mr. Sudhir Kumar

Academics

Registrar
Dr. Deepika Bhaskar

Technical Officer
Mr. Deepak Kumar

Management Assistant
Mr. Chakrawan Singh Chahar

Technical

Executive Engineer
Mr. R. K. Rathore

Technical Officers
Mr. Mahfooz Alam
Mr. Vijay Kumar Jha
Mr. Atin Jaiswal

Technical Assistants
Mr. Madhava Rao Medikonda
Mr. Suraj Tewari
Ms. Vishakha Chaudhary
Mr. Ramesh Chandiramouli
Mr. G. Nagavara Prasad
Mr. Kamlesh Satpute

Consultant (Scientific & Technical)
Dr. Nirpendra Singh

Consultant (Information Technology)
Ms. Alka Chug

Advanced Technology Platform Centre

Senior Technical Officer
Mr. S. Chandru

Technical Officer
Mr. Ashish Kumar Pandey

Software Engineer
Mr. Ajay Sehrawat

Executive Assistant
Mr. Amit Kumar Yadav

BSC BioNEST BIOINCUBATOR

Chief Operations Officer
Ms. Suman Gupta

Intellectual Property Manager
Ms. Mayuree Sengupta

Technical Assistant
Mr. Anshumouli Bhardwaj

Management Assistant
Ms. Ankita Srivastav

OFFICE OF CONNECTIVITY

Management Assistants
Mr. Yashpal Singh
Ms. Mahua Das


RCB
REGIONAL CENTRE FOR BIOTECHNOLOGY


Bio-technology innovation in the Health Sector-
March 1, 2018

REGIONAL CENTRE FOR BIOTECHNOLOGY

an institution of national importance for education, training and research

Established by the Dept. of Biotechnology, Govt. of India
Under the Auspices of UNESCO
3rd Milestone, Faridabad-Gurgaon Expressway
Faridabad - 121 001, Haryana, India
http://rcb.res.in