United Nations
Educational, Scientific and
Cultural Organization

REGIONAL CENTRE FOR BIOTECHNOLOGY

an institution of education, training and research

Established by the Dept. of Biotechnology, Govt. of India
Under the Auspices of UNESCO
180, Udyog Vihar, Phase 1, Gurgaon - 122016 India

Annual Report 2013-14

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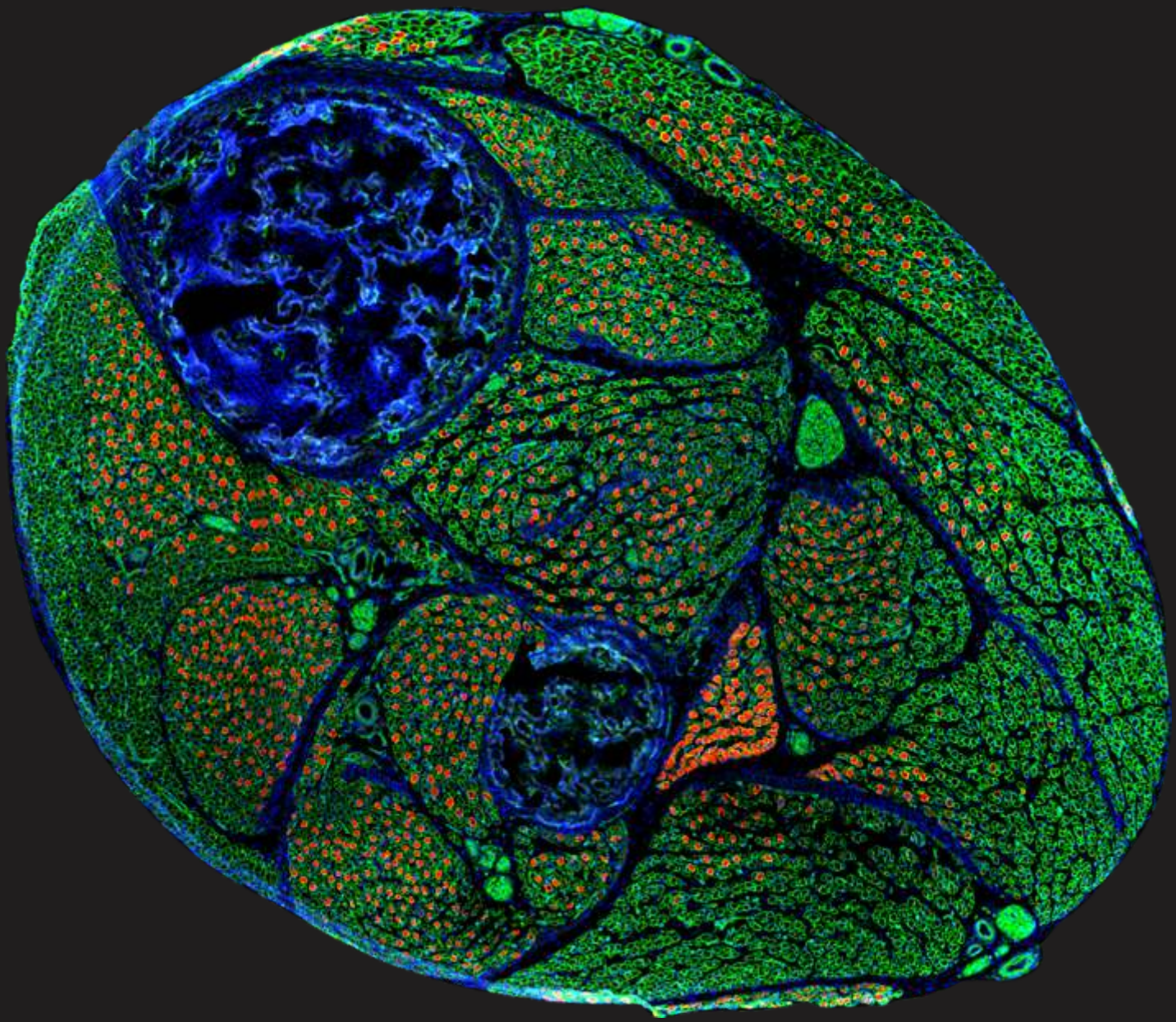


Contents

Contents

From the Executive Director's Desk

I am happy to bring out this report while the Centre is in the process of shifting to its permanent campus in Faridabad. For close to four years, functioning from the interim campus, the Regional Centre for Biotechnology has grown from strength to strength by adjusting to changing times and setting the highest standards.

The Centre is carrying out innovative research designed to create knowledge hub in biotech science. Centre currently has thirteen active research groups engaged in diverse activities within its mandate. In addition, RCB faculty are continuously engaged in attracting extramural grants from various organizations of repute for contemporary research at the interface of disciplines. Major intra-institutional and inter-institutional research programmes are being actively pursued. A multi-institutional study on pre-term birth involving a large cohort is currently involved in facilitating systematic exploration of the proteome to identify possible biomarkers has now been initiated for combating this problem.

Among the individual research programmes, the work on intrinsic signals that regulate skeletal muscle structure and function is focused towards understanding of how skeletal muscle differentiation happens during embryonic development as well as during adult muscle regeneration after injury or disease using animal models and in vitro techniques. The role of the cytoplasmic dynein subunit Light Intermediate Chain 2 (LIC2) in regulating mitosis in mammalian cell lines is being explored. LIC2 was found to be required for normal metaphase to anaphase transition through specific roles in inactivating the spindle assembly checkpoint, regulation of chromosome congression, spindle length and astral microtubule nucleation, and control of spindle orientation and spindle pole focusing.

Substantial progress has been made on the study of pathophysiology of thrombosis in disease conditions. Study also has proposed to explore the association of natural mutations of PHD2 with different physiological processes including erythropoiesis in high-altitude native Tibetans. Under the research programme on biology of infectious and idiopathic inflammation of the gut, results are being generated to understand the role of SUMoylation in inflammatory bowel disease and colon cancer. Another group studying on the complement regulatory proteins in autoimmunity illustrated that complement regulatory protein, called complement factor H (CFH), was majorly found to be present *ex vivo* on classical monocytes.

The research group working on understanding the molecular basis for function of ubiquitin pathway enzymes had revealed that the cancer-associated mutants of BAP1 induce the structural destabilization and subsequently undergoes beta amyloid aggregation. Research efforts are made towards unlocking the chemistry of cancer and nanomedicine. Studies are invloved in developing nanomaterials comprising drug delivery and DNA/siRNA delivery vehicles that would transform the cancer therapy by increasing efficacy of cutting down multiple pathways.

From the Executive Director's Desk

From the Executive Director's Desk

Structural biology approaches are being explored towards studies on antibody degeneracy illustrating crystallographic data of an antibody 2D10 complexed with a wide range of carbohydrate moieties. It had demonstrated that the antibody has adequate plasticity in interaction potential to accommodate a wide range of mono, di and tri-saccharides within a common binding site without significant structural changes. A research programme on structural investigations of *L. rhamnosus GG* (LGG) pilins, has progressed for detailed characterization and structure determination of two pilins (*SpaA* and *SpaD*). The studies on molecular determinants of genomic integrity and plasticity have provided insights into the initiation of replication in the RNA genome of the Japanese encephalitis virus. Structural biology investigations are also underway to understand the structure and activity of an anti-activator in the context of transcription regulation.

A programme on study of effector-triggered immunity in plants, wherein elucidation of inositol-regulated pathways in assemblies and signalling by immune regulators has been initiated, is progressing well. A previously identified inositol-biosynthesis Arabidopsis thaliana mutant altered in immune responses on the bacterial pathogen Pseudomonas syringae strain DC3000 is being is being characterized. Studies are also initiated to understand the mechanisms of how obligate biotrophic pathogens like the powdery mildew fungi modulate host plant immunity and nutrient allocation to fuel their own growth and reproduction.

The academic and training activities are making headway at remarkable pace. More than a dozen post-doctoral young scientists are being mentored at the Centre. Presently, more than 50 students are enrolled for the Ph D programme. It has become an important pillar of academic activities at RCB. Recently, the Centre has initiated design and development of a flagship course in Medical Sciences. This Masters level course shall cover concepts of Physiological Sciences, Immunology and Neurobiology and provide the clinically oriented students with an intellectually stimulating education in modern molecular, cellular and systems biology and neurosciences.

UNESCO has continued to support RCB in a variety of ways towards fulfilling its mandate for education, training and research in contemporary biotechnology areas relevant to India and the countries in this part of the world. Further, RCB is spreading its wings by collaborating with various national and international organizations of repute. In this context, its new partnership with the National Institute of Advanced Industrial Science & Technology (AIST), Japan will further strengthen capacity building initiatives in bioimaging and biotechnology. Domain-specific training programmes are also being organized in cutting-edge areas of biotechnology to open doors for new opportunities and to fill talent gaps in deficient areas, in partnership with national and international organizations such as IUBMB, IUCr and INSA, among others.

The faculty, younger scientists, students and the research staff engaged in various extramural research and academic activities have added a significant mass to the vibrant academic ambience of RCB. The dedicated technical and administrative staffs are continuously helping to create excellent institutional structures for facilitating fulfilment of RCB's mission. The encouraging support from the colleagues of the Department of Biotechnology, Govt of India, and that from the members of the Board of Governors, the Programme Advisory Committee, and various other Committees of the Centre have been vital for RCB to be where it is today. It is hoped that moving to the permanent campus in Biotech Science Cluster at Faridabad soon, would provide further impetus towards RCB's endeavour to be an institution of excellence synergizing scientific innovation with skilled human resource development.

Dr. Dinakar M Salunke
Executive Director

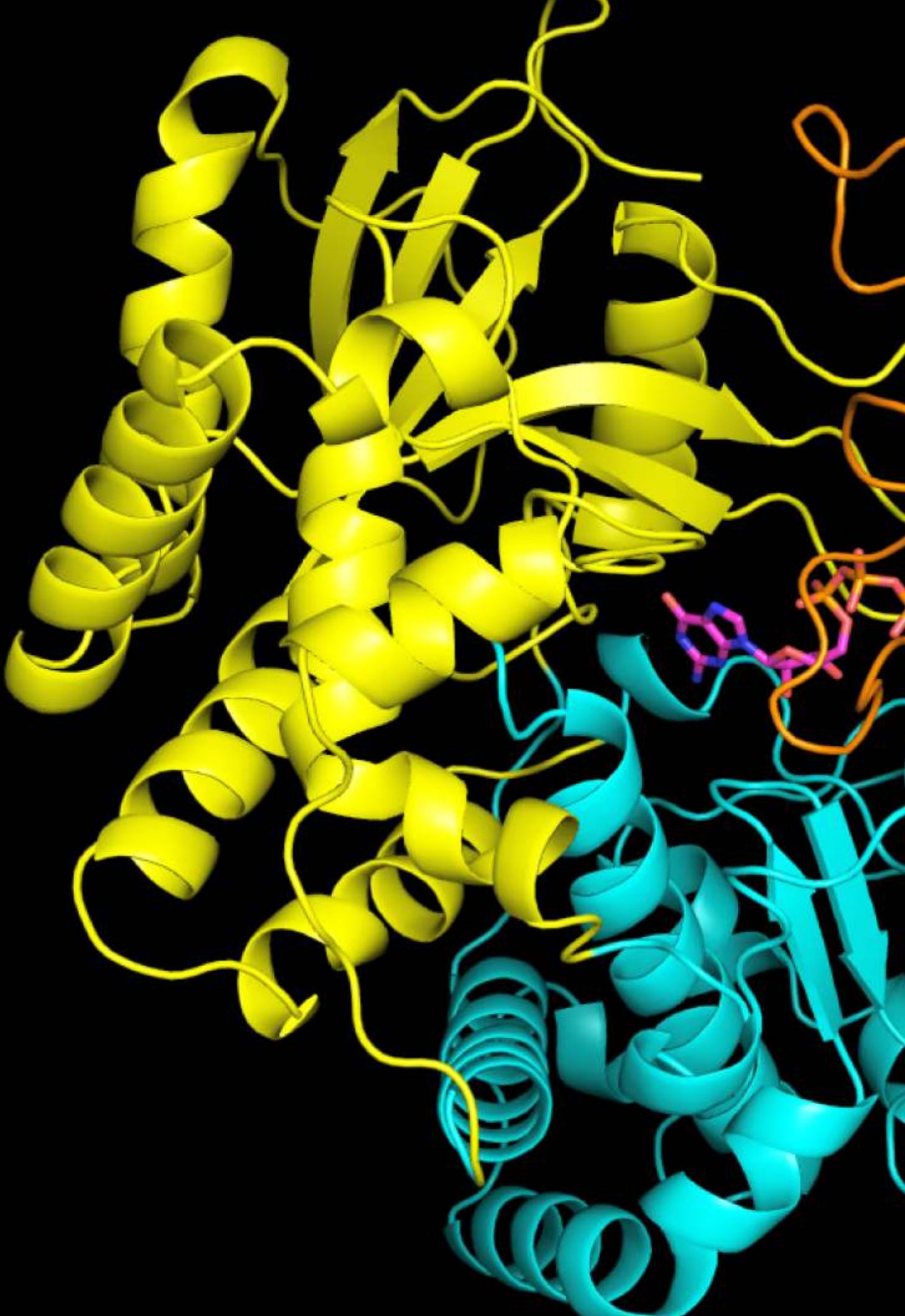
Mandate of the Centre

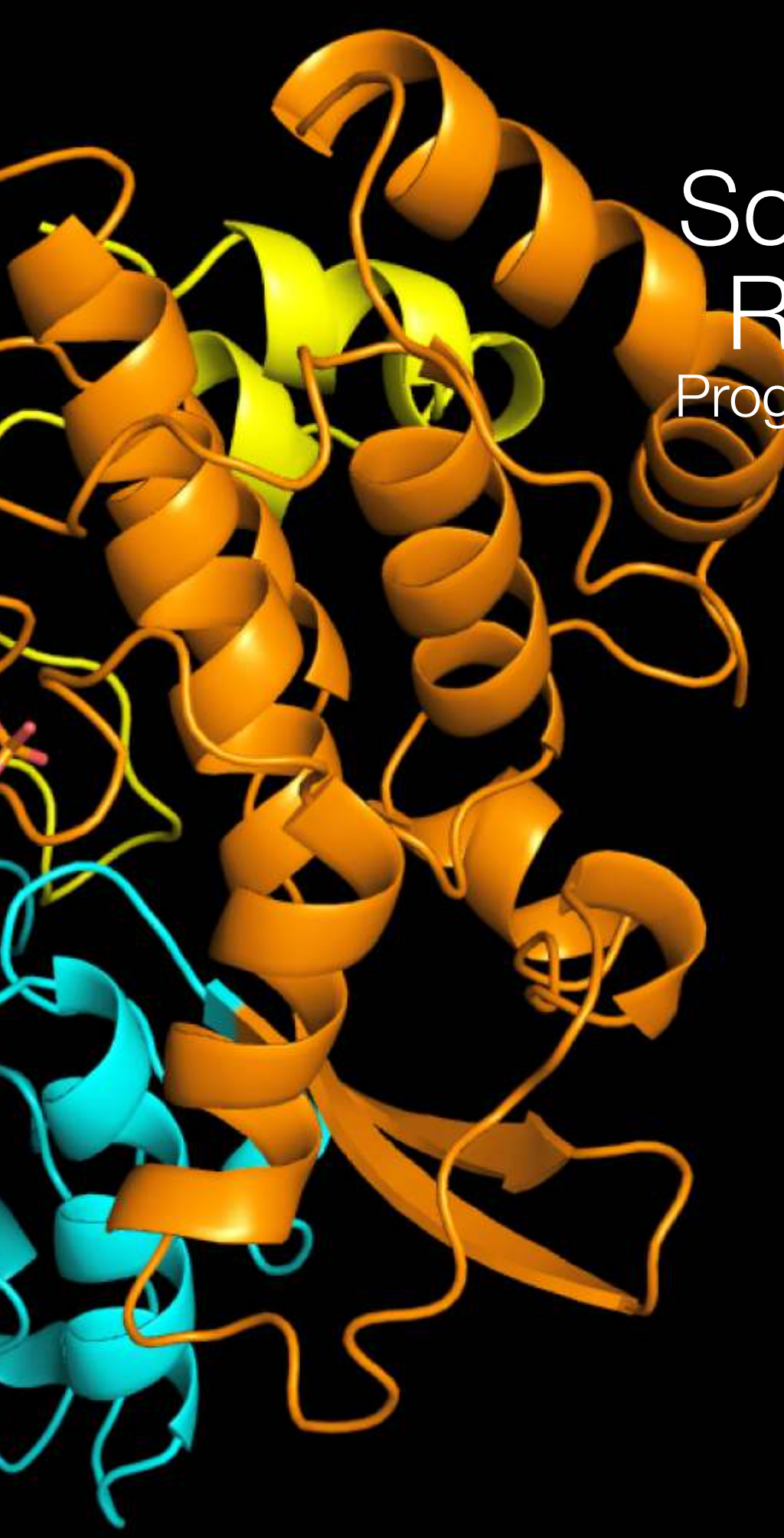
Mandate of the Regional Centre for Biotechnology is to provide a platform for biotechnology education, training and research at the interface of multiple disciplines. The programmes of the Centre are designed to create opportunities for students to engage in multi-disciplinary research where they learn biotech science while integrating engineering, medicine and natural sciences, to provide solutions for human and animal health, agriculture and environmental technologies. The vision is to produce human resource tailored to drive innovation in biotechnology, particularly in areas of new opportunities and also to fill talent gap in deficient areas. The Centre shall be an institution of international importance for biotechnology education, training and research (and shall, in due course, be constituted as an autonomous body under an Act of the Parliament). The Centre is regarded as a "Category II Centre" in terms of the principles and guidelines for the establishment and functioning of UNESCO Institutes and Centres.

The Centre functions with following objectives:

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

Mandate of the Centre





Scientific Reports

Progress during 2013-2014

Annual Report 2013-14

05

Regional Centre for Biotechnology
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Intrinsic Signals that Regulate Skeletal Muscle Structure and Function

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Research Theme

We are interested in understanding the process of cellular differentiation and its regulation using the skeletal muscle, by studying skeletal muscle development, and differentiation, by *in vivo* and in vitro approaches. We are also investigating the signaling events underlying tumors that exhibit characteristics of skeletal muscle cells.

Objectives

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

- Study the role of a family of skeletal muscle specific genes called skeletal muscle myosin heavy chains (MyHCs), critical to muscle structure and function, in development, and differentiation, using specific MyHC conditional targeted mouse alleles *in vivo*, and myogenic C2C12 cells *in vitro*.
- Document the temporal expression of MyHCs in vivo during development, and study the mechanisms underlying the Wnt-β-Catenin signalling pathway mediated regulation of MyHC expression and muscle differentiation.
- Identify genes with dynamic expression characteristics during C2C12 myogenic differentiation, and investigate their functional requirement in myogenesis.
- Investigate the role of the regulation of Met signaling in rhabdomyosarcoma, a tumor type wherein the tumor cells exhibit characteristics of skeletal muscle cells at various stages of differentiation.

Progress of work during the current reporting year 2013-14

The skeletal muscle Myosin heavy chain (MyHC) genes are a family of skeletal muscle specific genes, essential for muscle contractile function. There are multiple MyHCs and their expression is temporally regulated based on their functional relevance. Three such MyHCs, namely MyHC-embryonic, -perinatal and -slow are expressed by differentiating myogenic cells during embryonic development as well as regeneration in the adult. Mutations have been identified in all of these MyHCs, that lead to congenital syndromes such as myopathies and contracture syndromes like Freeman-Sheldon syndrome.

In order to investigate the role of skeletal muscle specific MyHCs during development and differentiation, we successfully generated a conditional targeted allele for MyHC-embryonic (MyHC-emb$^{fl/+}$) in mouse, the first MyHC to be expressed during development, by flanking exons 3 to 7 with LoxP sites (Figure 1).

Scientific Reports

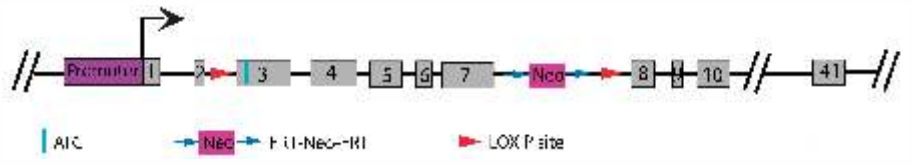

Figure 1. Schematic showing targeting strategy used to generate MyHC-emb$^{\Delta/+}$ animals. *The MyHC-embryonic genomic region depicting the promoter, exons (numbered grey boxes), introns (black lines connecting exons), start codon in exon 3 (blue line), LoxP sites flanking exons 3 ad 7 (red triangles), and the FRT-Neo-FRT cassette(blue triangles flanking box showing Neo).*

Using this allele, a knockout for MyHC-embryonic was generated (MyHC-emb$^{-/+}$), by crossing floxed mice (MyHC-emb$^{fl/fl}$) to HPRT$^{Cre/+}$ mice. Mice homozygous for the knockout allele (MyHC-emb$^{\Delta/\Delta}$) were generated by crossing heterozygous parents and we found that the frequency of homozygous mutant progeny was less than half that of the expected Mendelian ratio, suggesting that the MyHC-emb$^{\Delta/\Delta}$ genotype is semi-lethal in utero. We also verified that the MyHC-emb$^{\Delta/\Delta}$ allele is null for MyHC-embryonic, by immunofluorescence

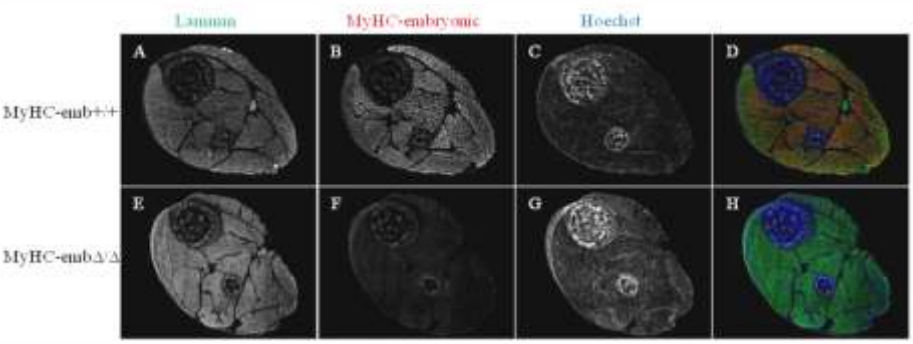

Figure 2. Confocal images of hind limb sections showing that MyHC-embryonic protein is absent in MyHC-emb$^{\Delta/\Delta}$ muscle, compared to MyHC-emb$^{+/+}$ muscle, by immunofluorescence. *Wildtype (A-D) and MyHC-emb$^{\Delta/\Delta}$ (E-H) P0 shank cross-sections immunolabeled for Laminin for basement membrane (A, E), MyHC-embryonic (B, F), and Hoechst for nuclei (C, G), showing absence of MyHC-embryonic in MyHC-emb$^{\Delta/\Delta}$ tissue.*

(Figure 2). All of the surviving MyHC-emb$^{-/-}$ animals (n=4) exhibited severe scoliosis or bent vertebral column by 6 weeks of age (Figure 3). Typically, the scoliosis affected the posterior part of the animals (Figure 3). It is interesting to note that scoliosis is a characteristic feature of the Freeman-Sheldon contracture syndrome, where missense mutations in MyHC-emb has been identified in patients.

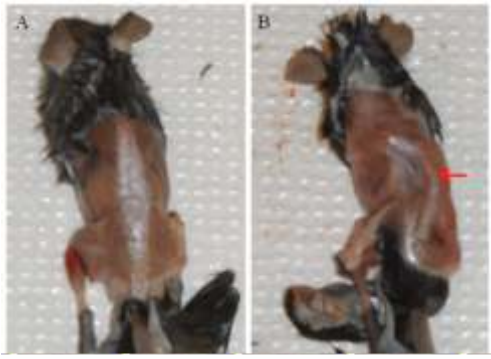

Figure 3. MyHC-emb$^{\Delta/\Delta}$ animals exhibit severe scoliosis. Wildtype (A) and MyHC-emb$^{\Delta/\Delta}$ (B) 10 week old siblings, showing the severe posterior scoliosis in the MyHC-emb$^{\Delta/\Delta}$ (B) animal (marked with red arrow). Patients with Freeman-Sheldon syndrome who have MyHC-embryonic mutations also exhibit scoliosis.

In an attempt to precisely establish the temporal sequence of protein expression of MyHCs during embryonic development, we performed a stage specific expression analysis by immunofluorescence using MyHC antibodies specific for different isoforms (Figure 4). Although this study is ongoing, preliminary results indicate that MyHC-embryonic is the first MyHC isoform to be expressed, with robust expression at E10.5, followed by MyHC-slow, which is also expressed at E10.5, and MyHC-perinatal, which is undetectable at E10.5 (Figure 4). Further characterization of earlier embryonic time points is required to obtain a better resolution to distinguish between expression of MyHC-embryonic and MyHC-slow. In addition, later embryonic developmental time points are also needed to establish when MyHC-perinatal expression is initiated.

We are also planning to identify genes involved in skeletal muscle differentiation, and are pursuing a candidate gene approach to this end. As a preliminary step, we screened by semi quantitative reverse transcriptase PCR (RT-PCR), co-repressor genes which have

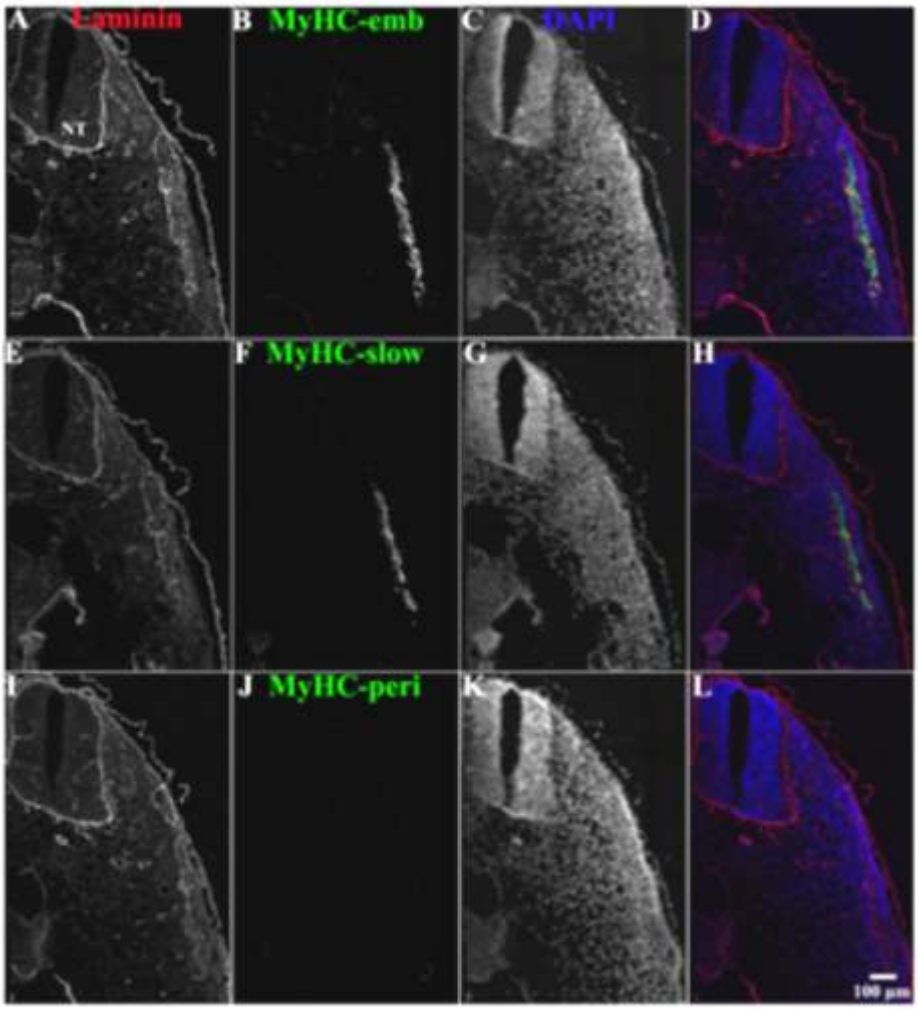

Figure 4. Confocal images of wild type E10.5 embryo sections showing protein expression of Myosin heavy chain (MyHC) genes. Adjacent sections from a wild type E10.5 embryo (A-D, E-H, I-L) immunolabeled for Laminin (A, E, I), MyHC-embryonic (B), MyHC-slow (F), MyHC-perinatal (J), DAPI for nuclei (C, G, K), and merged images (D, H, L), showing the temporal pattern of MyHC protein expression during development with MyHC-embryonic at highest levels, followed by -slow, and -perinatal.

Scientific Reports

been proposed in other systems to be involved in differentiation. One such gene is the Groucho co-repressor from Drosophila, for which there is a family of mammalian orthologs, called the TLE gene family, which includes 4 genes- TLE1, TLE2, TLE3, and TLE4. We plated C2C12 myoblast cells in 24 well dishes, allowed the cells to differentiate normally, harvested the cells at 24-hour intervals, isolated RNA, and generated cDNA from these samples. Using these cDNA samples, RT-PCR was performed to study TLE gene expression during myogenic differentiation (Figure 5).

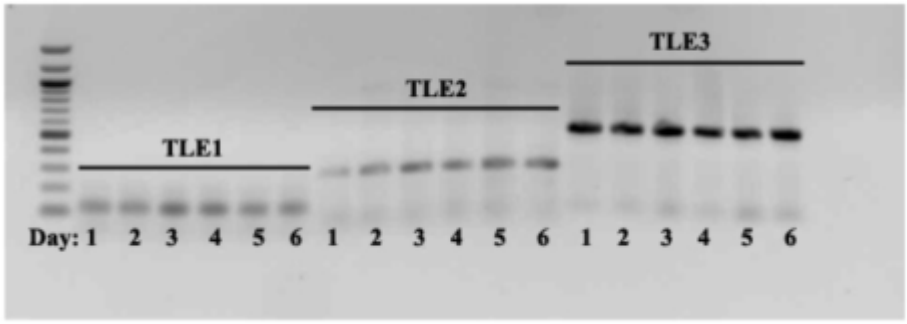

Figure 5. Semi quantitative RT-PCR showing gene expression of TLE1 and TLE2, with GAPDH as control, over 6 days of C2C12 cell myogenic differentiation. Agarose gel electophoresis of RT-PCR samples from cDNA generated from C2C12 cells allowed to differentiate over 6 days, with primers specific for TLE1, TLE2, and GAPDH. TLE1 levels seem to peak on days 3 and 4, whereas TLE2 levels increase with days of differentiation, while GAPDH (control) levels are constant through differentiation.

Preliminary results indicate that TLE1 levels peak on days 3 and 4 of differentiation, while TLE2 levels show a graded increase with differentiation, as compared to the control GAPDH. We are expanding this study to include TLE3 and TLE4 also.

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

Future plans

As part of the functional characterization of MyHC-emb$^{-/-}$ animals, we will investigate whether lack of MyHC-embryonic leads to aberrant developmental myogenic differentiation. We will also study whether the observed scoliosis in the MyHC-emb$^{-/-}$ animals is a consequence of developmental abnormalities or due to adult skeletal muscle defects. We will also initiate genetic crosses to generate animals with the correct genotype to perform conditional deletion of MyHC-embryonic during development. In addition, siRNA mediated MyHC-embryonic knockdown will be performed on C2C12 myogenic cells to understand the role of MyHC-emb on in vitro myogenic differentiation.We will also

expand these studies to include additional MyHCs, MyHC-perinatal and -slow, which are also developmental MyHCs similar to MyHC-embryonic.

In order to characterize the expression dynamics of MyHCs during embryonic development, we will continue our analysis of specific MyHCs using isoform specific antibodies for protein expression. In addition, transcript levels of MyHC isoforms will also be quantified during embryonic development by qPCR, using primers specific to each MyHC.

Our preliminary studies indicate that the TLE family of co-repressor genes are differentially expressed during myogenic differentiation and might be potential candidates that regulate differentiation. We will investigate this in greater detail by quantifying transcript and protein expression of the TLE genes by qPCR and western blots respectively using isoform specific primers or antibodies on C2C12 myogenic cell samples undergoing differentiation. Once the expression dynamics is ascertained, siRNA mediated gene knockdown studies will be initiated to study gene function of specific TLE isoforms during myogenic differentiation.

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

Molecular Determinants of Genomic Integrity and Plasticity

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Research Theme

We aim to elucidate the functional mechanism utilized by different molecular determinants of genomic integrity and plasticity. Our studies will provide insight into how organisms maintain their genomes and how variations arise in their genetic material.

Objectives

For all cellular processes to function optimally, the integrity of the genome has to be maintained. Conversely, plasticity in the genome can relieve selection pressure imposed by an adverse environment. These two conflicting requirements have led to the presence of molecules and pathways that either prevent (e.g. DNA mismatch repair) or facilitate (e.g. error-prone Polymerases) changes in the genome. The antagonistic action of these two different sets of molecules probably ensures that genomic plasticity is calibrated to endow adaptive capability without severely compromising genetic viability. In our laboratory, we intend to unearth the molecular mechanism employed by such molecular throttles of evolution to achieve function. With this broad aim in mind, the biological processes under scrutiny in my laboratory are (a) DNA Mismatch repair (b) Stress-Induced Mutagenesis (c) Translesion DNA synthesis (d) Replication of the Japanese Encephalitis Virus genome (e) Stress-induced epigenetic modification (f) Nucleotide Excision Repair and (g) Regulation of recombination (h) Transposition.

Progress of work during the current reporting year 2013-14

1. Replication of the Japanese Encephalitis Virus Genome

Japanese Encephalitis Virus (JEV) is a positive-stranded RNA virus and is the leading cause of viral encephalitis in the world. The viral genome encodes for three structural (C, prM and E) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). The NS3 and NS5 proteins form a tight complex and may represent the minimal replisome. A protease activity is present in the N-terminal region of NS3. The C-terminal region houses a RNA helicase activity that serves to melt secondary structures in single stranded RNA and unwind double stranded RNA templates for replication. A methyltransferase activity involved in cap formation is present in the N-terminal region of NS5. The C-terminus region

Scientific Reports

houses the RNA dependent RNA polymerase (RdRp) activity that is critical for replication of the viral genome.

Replication involves (i) initiation wherein the RdRP activity catalyzes ab initio RNA synthesis using a single-stranded RNA template to generate a dinucleotide primer (ii) elongation wherein the 3-OH on the primer is extended to generate a complement of the template strand. We aim to elucidate the mechanism of initiation and processive elongation. In addition, we want to understand how the multiple enzyme activities in the minimal replisome are coordinated to generate more copies of the single stranded RNA genome.

Viral RdRps exhibit low fidelity and consequently the viruses with RNA genomes exist in the form of a quasispecies. This attribute allows these viruses to present multiple genomic templates for natural selection to relieve selection pressure imposed by the immune response of the host. To understand how these viruses are pre-configured to evolve, we aim to unearth the source of error during replication of the JEV genome.

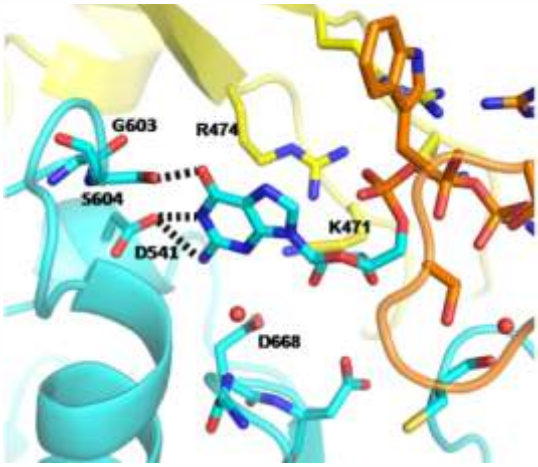

Figure 1. jRdRP forms specific interactions with the guanine base of GTP.

Unlike DNA Polymerases, the RdRp activity of many viruses does not require a primer and can initiate synthesis ab initio. In the case of Dengue virus, high concentration of GTP is required by the RdRp for initiation of replication. In a majority of the flaviviruses including JEV, the terminal dinucleotide sequence is 5' CU 3' and hence the requirement for GTP is surprising as GTP would be second incoming nucleotide.

We observed that, in the case of JEV RdRp (jRdRp), high concentrations of GTP (~100 µM) stimulates initiation but is not required for elongation. The jRdRpGTP complex structure (2.4 Å) provides a basis for the specific recognition of GTP (Figure 1). Using biochemical assays that probe for initiation activity, elongation activity and RNA binding affinity of native and mutant enzyme, we showed that the jRdRpGTP structure represents a previously uncharacterized pre-initiation state (Surana et al., 2014, NAR, 42:2758). GTP binding probably serves to partition nucleotide binding on either side of RNA binding to prevent unwanted and wasteful template independent RNA synthesis that could be erroneous. The proposed mechanism will enforce conservation of this functionally important terminal dinucleotide sequence. Also, the novel pre-initiation state that we have identified can be targeted by chemical therapeutics to combat the debilitating diseases caused by flaviviruses.

Scientific Reports

2. Translesion DNA Synthesis

The action of genotoxic agents leads to the presence of damaged nucleotides –termed as lesions- in the genome. These lesions inhibit replicative dPols and thus stall the replication

fork. It has been seen that members of the Y-family of dPols in all three domains of life (prokaryota, eukaryata and archaea) carry out translesion DNA synthesis to rescue stalled replication and prevent eventual death of the dividing cell. These enzymes utilize distinct strategies to achieve translesion DNA synthesis and their mode of action can be mutagenic. To unearth the structural and chemical strategies used by prokaryotic Y-family dPols to achieve translesion DNA synthesis, we are carrying out a rigorous biochemical and structural analysis of DNA Polymerase IV (PolIV)- a representative member of this family of dPols from *Escherichia coli*.

Error-free bypass of the 8-oxodeoxyguanosine lesion

The 8-oxodeoxyGuanosine (8oxoG) lesion arises due to the action of reactive oxygen species. The presence of this lesion in genomic DNA results in G:C to T:A transversions. These mutations appear because 8oxoG adopts the syn conformation that can form a Hoogsteen base pair with A and replicative dPols tend to incorporate an A opposite 8-oxoG. Steady-state kinetic experiments showed that PolIV can preferentially incorporate the C nucleotide opposite this lesion. Using a combination of macromolecular crystallography and steady state kinetic analysis of mutant and native enzyme, we have discovered the structural mechanism utilized by PolIV to mediate accurate bypass of this otherwise mutagenic lesion.

Error-free bypass of the minor groove N2-adducts

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

We observed that, unlike the Klenow fragment of dPolI, PolIV exhibits no difference in its ability to incorporate dCTP opposite template deoxyguanine and fdG. Steady state kinetic analysis showed that PolIV can incorporate dCTP opposite the fdG nucleotide with higher catalytic efficiency than undamaged dG. We have determined three structures of PolIV in complex with DNA containing N2-furfuryl adduct at the templating and two different downstream positions. These structures provide snapshots of the enzyme in the incorporation (Fig. 2) and extension modes during translesion bypass of the fdG adduct. The three structures show that the relative orientation of the fingers, palm, thumb domains and the PAD region allows the formation of an extended cavity that can be occupied by the furfuryl group for incorporation as well as extension modes. This set of structures- along with allied biochemical and functional data- allow formulation of a simple and elegant mechanism for the observed accurate translesion synthesis by PolIV past the replication-blocking N2-deoxyguanine adducts with high catalytic efficiency. Overall, this study provides the mechanism utilized by PolIV to neutralize the antimicrobial activity of NFZ and thus improve the viability of *E. coli* in the presence of this nitrofuran antibiotic. Due to high sequence homology (~ 60% identity), orthologs of PolIV from pathogenic bacteria may also subscribe to this mechanism to nullify the effect of NFZ and other nitrofuran antibiotics.

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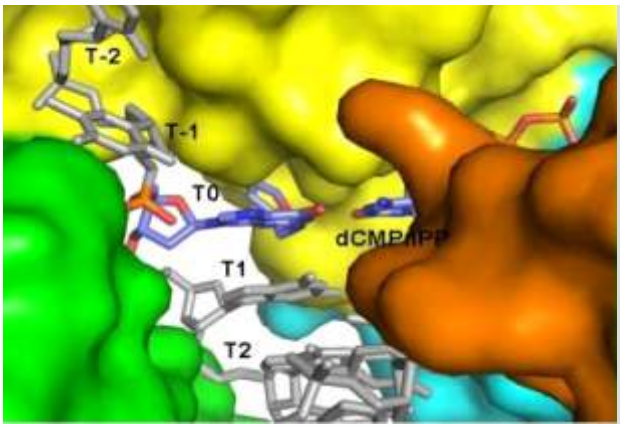

Figure 2. Close up of the active site of the PolI$_{YfdG:dCMPnPP}$ complex. The surface of fingers, thumb, palm and PAD domains are shown in yellow, orange, cyan and green, respectively. The fdG adduct is present at position T0 and is paired to the incoming nucleotide.

3. DNA Mismatch Repair

The Mismatch Repair (MMR) Pathway serves to maintain genomic integrity by correcting errors that appear during replication. In E. coli, the specific components of MMR are MutS, MutL and MutH. A majority of bacteria and all eukaryotes lack a homolog of MutH. It is, therefore, expected that these organisms will show significant differences in MMR especially in the mechanism of strand discrimination and nick-creation.

Using the pathway from *Neisseria gonorrhoeae* as a model system, we aim to elucidate the mechanism of MMR in organisms that do not follow the E. coli paradigm. The MutS and MutL homologs in *Neisseria* are named NgoS and NgoL, respectively. In addition to NgoL and NgoS, the processivity clamp-named Nβclamp- is thought to play an important role in strand discrimination. After extensive standardization, these three proteins can now be purified to high homogeneity.

The functional form of NgoS and orthologs is an asymmetric dimer. Two monomers associate to form an oval disc with a tunnel towards the bottom into which DNA is loaded to scan for mismatches. In the DNA-bound form, this tunnel of NgoS will be closed from all sides and therefore it is unclear how DNA is threaded through this cavity (Figure 3). The structure of NgoS in its apo- state shows that, in the absence of DNA, the clamp-connector domains of the two monomers do not contact each other (Figure 3). In addition, the N-terminal domain (NTD) is oriented away from each other resulting in the widening of the DNA channel. This results in the creation of an opening that will allow access to the DNA tunnel. The structure suggests that DNA enters the DNA tunnel through this opening. Following this, the clamp-connector (CC) and the N-terminal domains (NTD) reorient

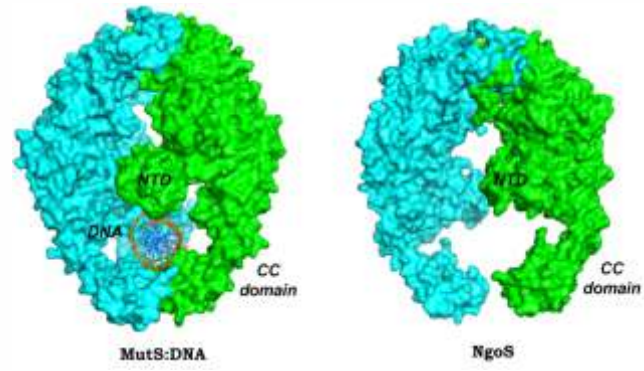

Figure 3. Comparison of the surfaces of DNA bound MutS (E. coli) and apo- NgoS.

to ensure that DNA is threaded through the correct tunnel. Overall, the structure of NgoS in the apo- state and allied biophysical studies reveal how a functional MutS-DNA complex is assembled.

4. Epigenetic modification to relieve environmental stress

There is an abundance of restriction modification (RM) systems in the gastric pathogen *Helicobacter pylori.* These RM systems regulate natural transformation and consequent genomic plasticity of this microbe. A number of DNA Methyltransferases (dMtases), that are part of these RM systems, show activity only in adverse conditions. The action of these dMTases results in the alteration of transcriptional profiles as a global strategy to deal with specialized environmental situations. We aim to elucidate the mechanisms that activate these enzymes under specific environmental conditions.

The expression of the HP0593 dMtase is upregulated when the pathogen encounters low pH. HP0593 is a TypeIII dMtase that belongs to the β class of these enzymes. The enzyme exhibits optimal activity at pH=5.5 and is predicted to modulate the expression of different genes in order to relieve acid stress. We have determined the structure of HP0593 in complex with the inhibitor sinefungin (SFG). The structure (Figure 4) coupled with biochemical and biophysical analysis of site specific mutants of HP0593 suggests that a functional dimer of HP0593 is formed only at low pH, thus ensuring that it is licensed to act only when the organism is subjected to acid stress.

Figure 4. Structure of HP0593: SFG complex at 2.75 Å

Future Plans

A clear mechanistic understanding of the activity of molecular determinants of genomic integrity and plasticity will provide deep insight into how these molecules impact the ability of an organism to survive and propagate in diverse environments. About 154 years ago, Darwin had postulated that new species arise through natural selection of genetic variations. Through studies on molecules that influence the appearance of these variations, we will contribute towards developing a deeper and more fundamental understanding of how organisms evolve. In the near and distant future, we aim to identify and characterize trans factors that regulate the activity of molecules that render genomic plasticity.

Genomic plasticity allows pathogens to develop resistance against therapeutic and

prophylactic agents. Perturbation of function of molecules that modify the genome will considerably potentiate the effect of available drugs and vaccines. Hence, the information that we derive from studies on molecular determinants of genomic plasticity will be used to discover chemical inhibitors that can serve as lead molecules for discovery of novel therapeutic agents. I also believe that these inhibitors can also serve as reagents to provide deeper insight into the mechanisms employed by target enzymes to achieve function. Overall, we will employ and develop methods to exploit the translational value of discoveries made in the laboratory.

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

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Research Theme

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

Objectives

Protein posttranslational modifications (PTM) such as phosphorylation, ubiquitination, SUMOylation, redox-modification, acetylation and glycosylation play an important role in different cellular processes including protein quality control, cell cycle regulation, endocytosis, DNA repair, vesicles trafficking and so on. Dysregulation of these processes lead to different diseases like cancer. It is well established that protein ubiquitination is one of the most important PTMs, which regulates almost all cellular processes. One of the important aspects of our research is to understand the ubiquitin signaling mechanism and their regulation in cellular pathway and diseases. Human genome analysis and proteomics data reveal almost one hundred deubiquitinating enzymes, which majorly regulate the ubiquitin homeostasis in cells. However the molecular functions of most of the DUBs are still nonelusive. We are investigating the molecular basis of their involvement in cellular functions like protein degradation, histone modification, and endocytosis of plasma membrane proteins. It has also been revealed that the dysregulation of deubiquitinating enzymes lead to diseases like parkinson, alzheimer, ataxia, heart disease and different types of cancer. Our aims are to understand the possible molecular mechanism underlying these diseases.

Progress of work during the current reporting year 2013-14

Since last three decades a considerable progress has been made in the genome sequencing which revealed the genomic landscape of cancer. The cancer genome studies have shown that there are more than hundred genes altered due to intragenic mutation, which may drive tumorgenesis. In the specific tumor type there are some driver genes, which regulate the core cellular processes like cell fate, cell survival and genome integrity. In recent years, BRCA1 associated protein 1(BAP1), a nuclear deubiqutinating enzyme which has emerged as an important tumor suppressor protein, undergoes frequent mutations in different types of cancer and appears as one of the important driver genes in many cancer types like uveal melanoma, mesothelioma, renal cell carcinoma, cholangiocarcinoma and melanocytic tumors. Human BAP1 is a 729 amino acids of multi domain deubiqutinating enzymes whose N-terminal domain (1-240) shows homology to other ubiquitin C-terminal hydrolase (UCH) enzymes. The C-terminal domain mediates

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binding to the N-terminal RING of BRCA1 and regulates the BRCA1 mediated tumor suppressor function. BAP1 also regulates E2F mediated cell cycle regulation through the direct interaction of Host cell factor-1. It has been shown that BAP1 is a part of PR-DUB complex where it interacts with ASXL1 and regulates epigenetic function by removing ubiquitin from H2A.

While BAP1 and its involvement in cancers are most typically caused by the mutations that lead to the loss of protein function or deletion of key regulatory domains, the missense mutations are more prevalent in the tumors. The COSMIC database showed that out of 108 disease-associated mutations identified 69 are located in the catalytic domain of BAP1 and almost 60% of the missense mutations are localized to catalytic domain.

The major aspect of our research is to understand the BAP1 mutation and its impact on cellular processes. It is presumable to believe that mutation of any gene has severe consequence in its function. Using the biochemical analysis of catalytic domain containing five mutants of BAP1, we have demonstrated that mutation in the catalytic domain impaired the enzymatic activity, significantly loss of protein stability and subsequent protein aggregation. The protein structure destabilization and subsequent aggregation play a profound role in numerous human diseases specifically neurodegeneration and cancers. Alteration of protein structure occurs due to the genetic mutation or predisposition of genetic elements leading to the deactivation of protein function. Uncontrolled overexpression or structural instability caused by the mutation contributes to gain of toxic function and shows the dominant negative effect. There is accumulation of evidences that missense mutation, where a single change of amino acid plays an important role in tumor

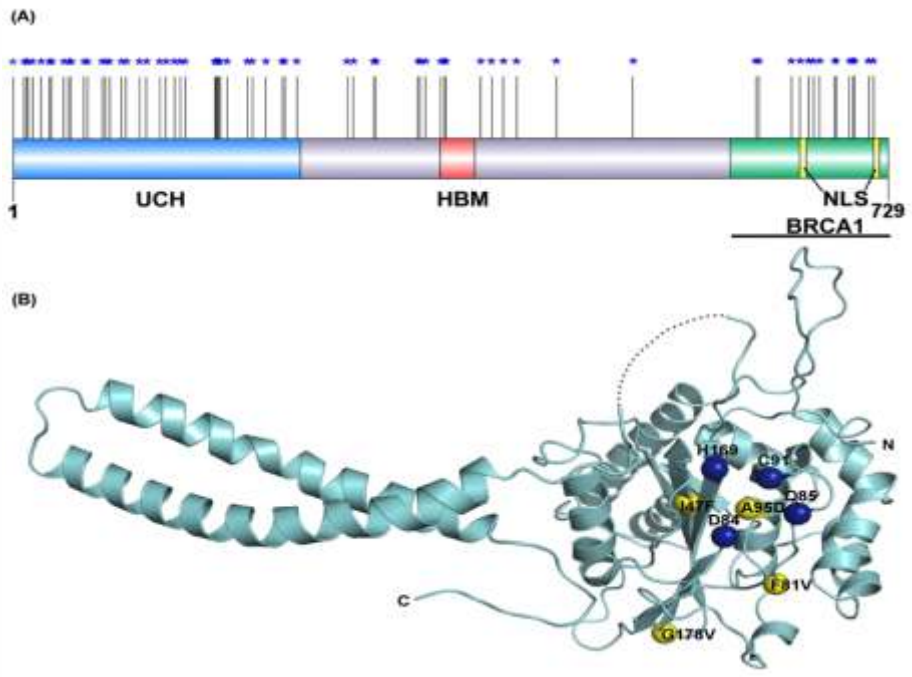

Figure 2. Dot blot analysis of BAP1 and all cancer-associated mutants. BAP1 wild type and mutant protein were overexpressed in HEK293T cells, homogenized, and subsequently loaded onto Superose 6 column. Each column fraction was tested with anti-BAP1 and Anti A11 antibodies.

progression. However, it remains unexplored how protein aggregation caused by alteration of protein structure particularly oncogene or tumor suppressor leads to the induction of malignancy. In the present report we have tried to demonstrate the molecular basis of the inactivation of BAP1 mutants and possible link in cancer mechanism.

Sequence alignment of all UCH members shows that there are several amino acids conserved in the UCH domain which are conserved from flies to human indicating that they are critical in the protein structure and function. The domain wise distribution of all missense mutant of BAP1 in cancer and the location of the mutation in UCH domain are given in the Figure 1. Earlier studies reported that A95D and G178V mutations impaired the catalytic activity. However the molecular basis of inactivation has not been elucidated. We have included another two cancer associated mutants I47F and F81V which are also prevalent in many cancers. A95D position is close to catalytic cysteine residue and in the same helix (Figure 1) whereas I47F, F81V and G178V mutants are away from catalytic site. The crystal structure of BAP1 is yet to be solved and homology model structure predicts that I47F in the beta 3 and F81V and G178V are residing in the loop region.

To understand how these single amino acid changes cause protein function, we introduced each mutation HA-FLAG-BAP1 by site directed mutagenesis. To investigate the effect of structural mutations in BAP1 on cellular function we first transiently overexpressed wild type and mutant BAP1 in human lung squamous carcinoma cell line NCI-H226, which is devoid of endogenous BAP1. The immunofluorescence using confocal microscopy showed that there is more cytoplasmic accumulation/less nuclear localization and more perinuclear localization of BAP1 in the mutants compared to wild type protein. This result suggested there is an impairment of nuclear transport. The perinuclear localization might be due to the aggregation of BAP1. To address the oligomization/ aggregation status of BAP1, we have transiently overexpressed BAP1 wild type and I47F, F81V, C91S, A95D and G178V in HEK293T cells. All mutants showed similar efficacy in invitro cellular condition. The cells were harvested and dissolved in buffer containing 50mM Tris-HCl, 150mM, NaCl with 0.5% sodium deoxycholate and protease inhibitor. Equal amount of clear cell lysate (~0.4mg) was loaded onto a Superose 6 (10/300 GL) gel filtration column, which is equilibrated buffer containing in 50mM Tris-HCl, 150mM NaCl, 5mM DTT and 5% glycerol. Each chromatographic fraction was tested in a dot-blot using anti BAP1 and anti A11 antibodies (Figure 2).

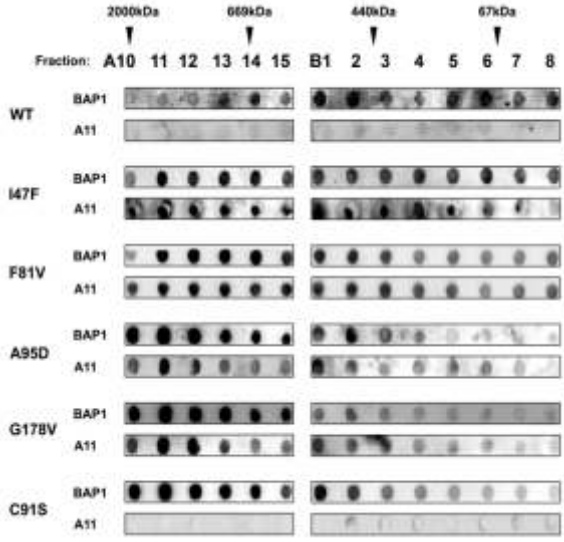

Figure 2. Dot blot analysis of BAP1 and all cancer-associated mutants. BAP1 wild type and mutant protein were overexpressed in HEK293T cells, homogenized, and subsequently loaded onto Superose 6 column. Each column fraction was tested with anti-BAP1 and Anti A11 antibodies.

Our dot-blot analysis showed that the wild type protein eluted

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in the wide range of molecular weight from 700kDa to 70kDa due to the formation of multi-protein complex. On the other hand all mutants eluted from void volume to high molecular weight (700Kda). Conversely wild type protein did not elute in the void volume indicating that the mutant BAP1 formed of large aggregates in cellular condition. To characterize the aggregates we have tested the reactivity with A11 antibody, which specifically reacts with prefibrillar amyloid aggregates. Wild type and catalytic inactive mutant C91S did not show reactivity towards A11 on the other cancer associate mutants showed very high reactivity with A11. Our result unambiguously demonstrated that catalytic domain mutants of BAP1 showed amyloidogenic behavior.

For better understanding the aggregation property, the His-tag BAP1 (729) was cloned; expressed and purified though the expression of full-length protein in *Ecoli* expression system is considerably low. All biochemical, biophysical and enzymatic experiments have been carried out using catalytic domain of BAP1 (1-240). All our mutational analysis was carried out in the catalytic domain protein. To examine the invitro effect of mutation on the enzymatic activity we have performed enzymatic assay using a model substrate, Ub-AMC. The BAP1 (1-240) showed almost 1000 fold higher activity compared to the BAP1 (1-729). All mutants have shown complete loss of enzymatic activity (Figure 3). To examine the effect of mutation on the secondary structure of BAP1 catalytic domain, we analyzed WT and mutant proteins using circular dichroism spectroscopy. The secondary structural analysis by CDSSTR showed that I47F, F81V and A95D mutations induced almost 5-10% increase in beta sheet formation.

In order to understand the effect on the stability of the protein we performed the thermal melting analysis of WT and mutant BAP1. The cancer causing mutation induces the thermal instability, which is reflecting in the Tm values. Wild type BAP1 (1-729), BAP1 (1-240) and all the mutants except A95D showed sharp transition and lowering of Tm (Figure 3). Surprisingly A95D showed swallow transition with melting temperature ~60^{0}C and appeared to unfold in noncompetitively. The secondary structure did not collapsed completely even after heating upto ~85^{0}C. This data provided us a clue for specific orderly aggregate formation by A95D mutant.

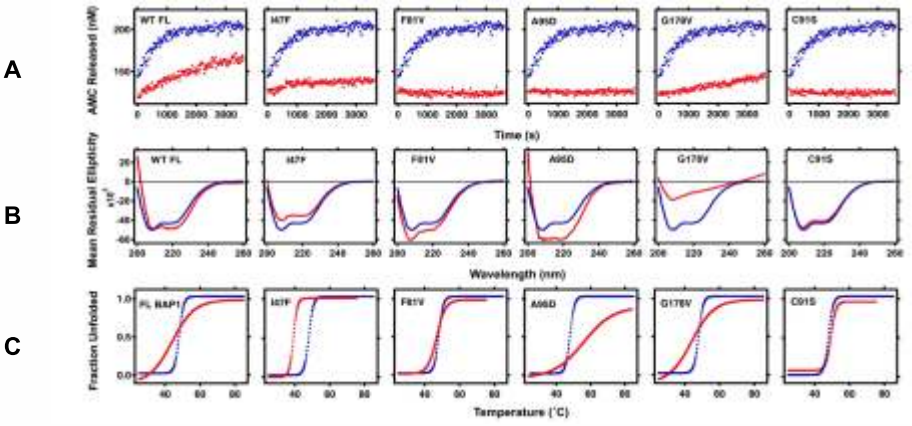

Figure 3: (A) Enzymatic activity of BAP1 and mutant proteins. Blue and red dots represent the progress curve of Ub-AMC hydrolysis by wild type and mutant BAP1 respectively. (B) Circular dichroism spectra of wild type (blue) and mutant (red). (C) Thermal melting of wild type (blue) and mutant (red) BAP1.

To better understand why structurally destabilized mutations in BAP1 would induce aggregation, we used TANGO, an algorithm for predicting protein aggregation sequences, to identify regions in the protein that would be prone to aggregation. Here we have identified a few aggregation prone segments that span from residues 13-20, 43-52, 78-100 and 656-680. The 43-57 segment shows highest tango score representing maximum propensity of beta aggregation. The homology model structure of BAP1 catalytic domain shows that the aggregation prone regions are lying in the hydrophobic core of the protein. Mutations that destabilize the tertiary structure of the catalytic domain are likely to increase the exposure of the regions that are normally buried in the hydrophobic core, such as the aggregation-nucleating region. So these mutations are also prone to trigger aggregation of the BAP1 by assembly of the beta amyloid type aggregation into an intermolecular structure.

We measured the binding of the thioflavin-T dye, which binds specifically order aggregates with BAP1 wild type, and all mutants at 25^{0}C and 37^{0}C heating followed by 2h incubation at 25^{0}C. The ThT binding results showed that all mutants bind preferably with ThT dye compared to wild type protein. Though BAP1-FL has considerably ThT binding at 37^{0}C heating followed by 2h incubation at 25^{0}C compared to catalytic domain protein indicating that there is possibility of inherence amyloidogenic character of BAP1 protein. The congo-red binding data also suggested that BAP1 mutants more specifically bind with congo-red dye, which binds more specifically beta amyloid protein. The beta fibril formation by the mutants also confirmed by atomic force microscopy (AFM) (Figure 4). The AFM results indicated that A95D, I47F, F81V and G178V undergo fibrillar aggregation upon heat induction at 37^{0}C. To our surprise BAP1-FL also showed a very nice febrile upon heat induction. It is evident from Tango score analysis that BAP1 has several beta propensity regions mostly in catalytic region and ULD. Consensus structure prediction also revealed that the middle region of BAP1 is mostly unstructured. Binding with interacting partners might bring this region more stable and structured. This might be the probable reason for BAP1's beta aggregation upon heating.

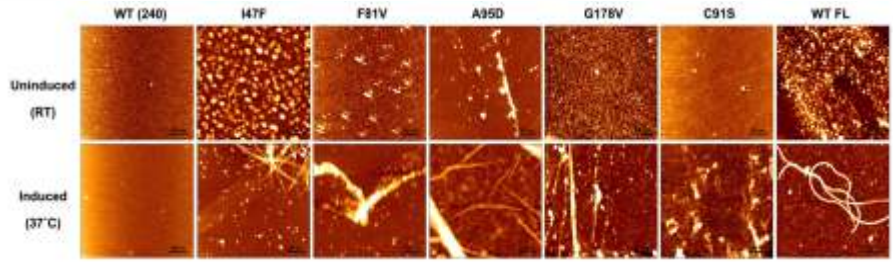

Figure 4: Atomic force microscopy image of wild type and mutant BAP1. (A) AFM image at 25^{0}C temperature (B) AFM image at 37^{0}C heat induced condition.

How BAP1 aggregation is associated with the cancer pathogenesis is the subject of our future study. We are investigating the how these aggregation alter the cellular pathways by integrated approaches like chaperone function, ER aggregation protein response.

Future plans

System biology approach to understand the cancer causing mutations observed in different cancers will be the attractive strategy for molecular analysis of cancer. iTRAQ label based differential mass spectrometric-based proteomic approach will be adopted to elucidate the molecular mechanism. We will overexpress different BAP1 mutants and wild

type protein in BAP1 knockout cell line NCI H226 (no endogenous expression). Proteomic and subsequent pathway analysis will provide a clue for passible mechanism of BAP1's involvement in cancer.

BAP1 interacts directly with many proteins like BRCA1, BARD1, ASXL1, OGT, HCF1 and YY1, which are major players in different cellular processes. Thus structural analysis of BAP1 will provide mechanistic detail of its cellular function. We are trying to crystallize BAP1 (1-240) catalytic domain. Domain wise truncations of BAP1 are already made and ready for protein expression and purification. Catalytic domain of Calypso (1-315), a drosophila analog of BAP1 has been cloned in pGEX-6P1 vector and the purification of this protein is under process. Ecoli expression system does not overexpress BAP1 (1-729) protein hence we have initiated a baculovirus expression system for purification.

The sequence alignment of BAP1 with other UCH member proteins showed sequence conservation ranging from 24% -63% (UCHL1 (24%), UCHL3 (26%) and UCHL5 (43%) and Calypso (62%)). We are aiming to replace UCH domain of BAP1 (1-240) with other UCH members. We like to investigate the cellular property of BAP1 like cell cycle regulation and Histone 2B deubiquitination and ubiquitin chain processesivity and interaction with interacting partners. The thermodynamics of interaction of BAP1 with ubiquitin and the comparative study with UCHL1 UCHL3 and UCHL5 have been undertaken.

Transcription Regulation: Structure and Mechanism

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Research Theme

Using an integrated multidisciplinary approach, our laboratory addresses the molecular mechanism of transcription and its regulation in different physiological contexts. We investigate molecular interactions (protein-DNA, protein-small metabolites, protein-protein) that are critical for regulating transcription.

Objectives

We aim to elucidate:

- The mechanism utilized by bacterial enhancer binding proteins and their associated factors to regulated transcription using regulation of flagellar genes expression in *Pseudomonas aeuroginosa* as a model system
- The mechanism utilized by single subunit plastid RNA polymerases for initiation of synthesis and elongation of the mRNA transcripts using the RpoTp enzyme from *Arabidopsis thaliana* as a model system
- The allosteric mechanism that renders transcription factors responsive towards small metabolites using the arabinose-sensitive regulator AraR from *Bacillus subtilis* as the model system.
- The mechanism of stress-induced crosstalk between two component systems using the VraS and GraS signalling circuits in *Staphylococcus aureus* as the model system

Progress of work during the current reporting year 2013-14

A) Structural mechanism utilized by bacterial Enhancer Binding Proteins (bEBPs) to regulate sigma 54-dependent transcription initiation

Since their discovery, it has become evident, that sigma factors are central to the function of the RNAP holoenzyme. There are two evolutionary distinct families of sigma factors in bacteria. The primary sigma factor referred to as σ70 and the σ54. Most of the genes required for growth in the exponential phase are expressed by primary sigma factor. It has the highest affinity for core RNAP and is also the most abundant sigma factor throughout the growth cycle. Many bacteria also code for alternative sigma factors

Scientific Reports

which regulate specific sets of genes under various stress conditions. These alternate sigma factors also belong to different sub groups of the σ70 family.

σ54 (RpoN), on the other hand possess several unusual features. Unlike the primary sigma factor, σ54-RNAP forms a stable closed complex and is unable to melt DNA to form transcriptionally active open complex. Isomerization occurs only when the σ54-RNAP complex interacts with an activator that hydrolyzes ATP (Figure 1). These activators typically bind to specific sequences located 100s of bases upstream of the promoter. Consequently, the interaction between σ54-RNAP and the activator causes the intervening DNA to loop. Since the activator resembles the enhancer binding proteins (EBP) of eukaryotes they are known as bacterial EBPs (bEBPs).

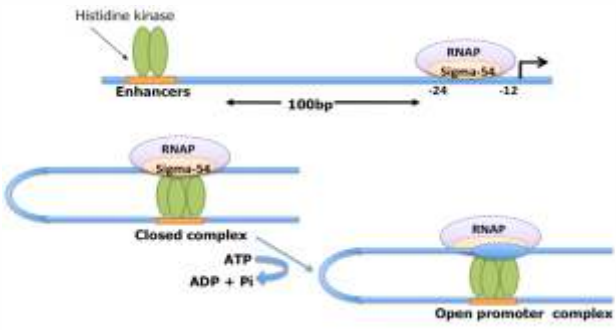

Figure 1. Schematic showing steps involved in sigma54 dependent

All bEBPs, like the eukaryotic transcriptional activators have a modular structure consisting of three distinct functional domains (Figure 2). The N- terminus domain serves as a target for regulatory signals. The C-terminus DNA binding domain consists of a helix-loop-helix motif and recognizes the UASs. A central domain that interacts with the σ54 is responsible for transcription activation and ATP hydrolysis. Although structures of various domains of σ54-dependent activators and CryoEM reconstruction of one of the activator bound to RNAP is available, the information on nature of conformational changes that occur in the activator on RNAP binding is lacking. Also it is not clear as to how the DNA melting is triggered at σ54-dependent promoters. What are the structural features of the σ54-RNAP open promoter complex? In order to answer these questions, we will employ FleQ, a master regulator of flagellar and biofilm genes in *Pseudomonas aeruginosa* (Psa) as a model system. FleQ controls the expression of flagellar genes in a σ54 dependent fashion and is present at the apex of the flagellar transcription cascade. Deletion or mutation of FleQ gene makes the bacterium non-motile.

Figure 2. Domain organization of FleQ

The activity of FleQ is in turn regulated by a protein called FleN. FleN is a putative ATP/GTP binding protein that interacts directly with FleQ without affecting its DNA binding ability. Mutation of *flen* results in a multiflagellated bacterium which shows chemotactic defects due to upregulation of flagellar genes. Thus, FleN acts as an antiactivator that regulates flagellar numbers by regulating the activity of FleQ. In order to obtain mechanistic insights into the modes of transcription regulation by FleN, we are studying the molecular interactions between FleQ and FleN. To begin with, we have

determined the crystal structure of FleN in complex with non-hydrolysable analog of ATP (AMPPNP) at 1.55Å resolution.

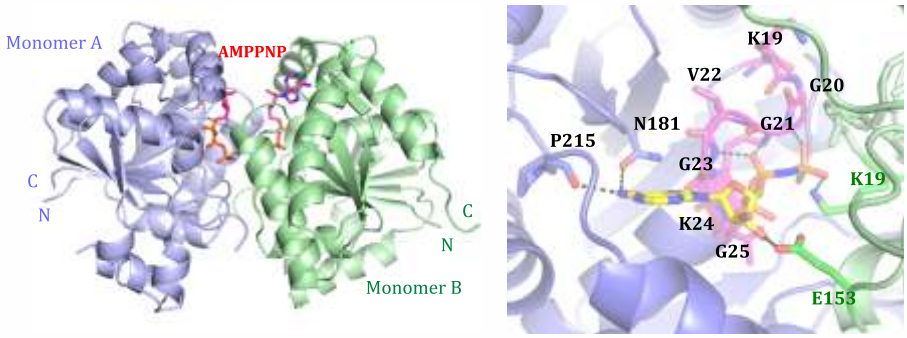

Figure 3. Crystal-structure of FleN/AMPPNP complex. (A) Dimer of FleN binds to AMPPNP through the walker A motif. (B) Details of interactions of AMPPNP bound to FleN

We have also determined the structure of FleN in apo state at 2.6Å resolution. On the basis of the crystal structure, we have identified mutations of FleN that will perturb the dimer. These mutants have been purified and currently DLS experiments and functional assays are ongoing to charaterize the AMPPNP mediated dimerization of FleN.

A) Mechanism of transcription initiation and elongation by RpoTp: The single subunit RNAP from *Arabidopsis thaliana*

RpoTp is localized in the chloroplast and is involved in transcription of plastid genes. It has been proposed that this enzyme transcribes genes encoding components of the plastid genetic system such as those of translational apparatus and core subunits of multisubunit RNA polymerase. RpoTp can initiate transcription at different promoters on the plastid genome and is expected to be regulated by different uncharacterized transcription factors. Currently, we have cloned, expressed and purified RpoTp. We will perform the invitro transcription assay to check the activity of the purified enzyme. Subsequently, the enzyme will be subjected to crystallization trials to obtain structures of this enzyme in its apo state and bound to promoter DNA. We will also attempt to crystallize the transcription elongation complex comprising of the RNAP, double stranded DNA, RNA and incoming nucleotide. Additionally, we will use purified RpoTp to initiate proteomic approaches that will identify novel regulators of RpoTp that modulate its transcription activity at different promoters.

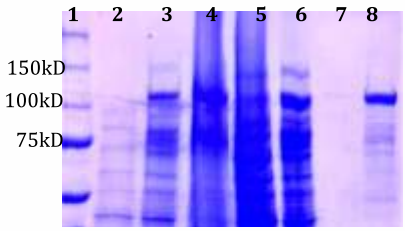

Figure 4. SDS gel showing purification of RpoTp

B) Allosteric mechanism utilized by transcription factors responsive towards small metabolites

Transcription factors modulate gene expression primarily through specific recognition of cognate sequences resident in the promoter region of target genes. Transcription factors bind not just one but several different target sites to modulate gene expression. AraR protein is the key regulatory protein of the L-arabinose metabolism in *Bacillus subtilis.* AraR binds to eight different operator sequences governing five different promoters and has two different modes of transciptional repression. At metabolic operon and araE promoters the AraR operators are present in pairs (ORA1:ORA2, ORE1:ORE2 and ORX1:ORX2) and the repressor binds cooperatively to these sites with a dimer of AraR binding to each operator. This results in DNA bending between the operators, as a result the promoter becomes inaccessible to the RNA polymerase thereby leading to enhanced down-regulation of transcription. On the other hand AraR dimers can also bind non-cooperatively to single operators such as ORR3 and ORB1 without DNA looping and resulting in non- stringent repression.

AraR, is composed of two independent domains exhibiting different functions and belong to different family of proteins. The smaller N-terminus domain (NTD) which retains its ability to bind DNA, comprizes winged helix-turn-helix motif and the larger C-terminus domain (CTD) binds L-arabinose and belongs to LacI/GalR. In the absence of L-arabinose, AraR binds to operator sequences as a result the metabolic genes cannot be transcribed. Presumably, AraR undergoes a conformational change on binding L-arabinose which releases it from cognate operators resulting in transcription initiation.

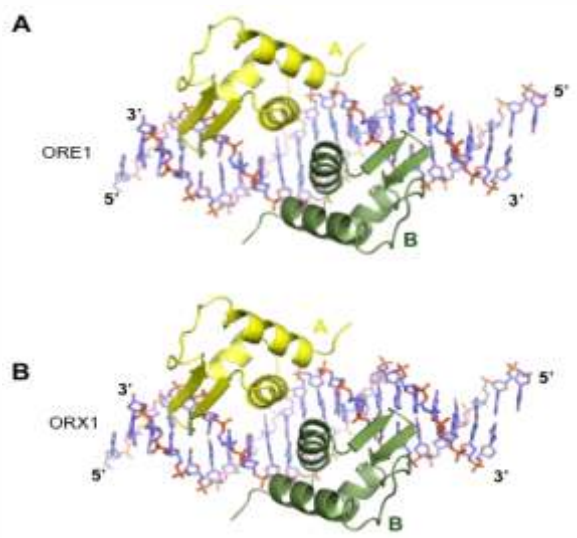

Figure 5. Crystal structures of AraR-(NTD) bound to operators (A) ORE1 and (B) ORX1

We have earlier determined the X-ray crystal structure of NTD of AraR bound to two natural operators, ORA1 and ORR3. Two AraR-NTD molecules are bound to each operator with one monomer interacting with TXG motif present in one half site of the operator. The structural data revealed that the binding mode of AraR to DNA is dependent on the spacing between the two TXG motifs in the two half site of the operator and the protein undergoes drastic changes with respect to its binding position on DNA. Recently we have determined the crystal structure of AraR-NTD in complex with two other natural operators ORE1 and ORX1. The structures show the specific protein-DNA interactions used in each case for operator binding. The interaction of AraR-NTD with ORX1, which lacks the TXG motif in one half site are altered from the interaction seen in case of AraR-NTD bound to TXG

motif in the second half site. Comparison of interactions of AraR-NTD to all the four operator sequences suggests that breakdown of symmetry in the operator sequence is compensated by plasticity of the repressor for DNA binding. Finally we have also determined the binding affinities of full length AraR as well as AraR(DBD) to these operators using fluorescence anisotropy.

Currently, efforts are underway to obtain the crystals of full length AraR bound to DNA and L-arabinose. These data will provide insights into the structural basis for the conformational switch in AraR that abolishes specific DNA recognition on arabinose binding.

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

The two component systems are signalling pathways that enable bacteria to sense and respond to diverse ranges of environmental signals such as pH, nutrients, antibiotic stress etc. A typical two component system comprises of a sensor histidine kinase (S) and a cognate response regulator (R). The histidine kinase receives the signal that activates the system, whereas the response regulator is often a DNA binding transcriptional regulator. The activation domain of histidine and the receiver domain of the response regulator are widely conserved as a result the histidine kinase of one regulatory system can sometimes, activate the regulator of another. This phenomenon is known as "cross-talking of two component systems.

The VraSR (vancomycin resistance associated) system is a typical two component system in *Staphylococcus aureus*, where VraS is the sensor histidine kinase and VraR is the response regulator. It was observed earlier that the inactivation of VraS, resulted in increased tolerance to glycopeptide antibiotics like vancomycin raising a possibility that the response regulator was being activated by an alternative kinase possibly GraS. GraS is part of the GraSR (glycopeptide resistance associated) two component system. We will carry out a rigorous investigation of the interactions responsible for cross talk between the VraSR and GraSR regulon. Cloning of the relevant genes is currently ongoing. The potential protein-protein interaction between GraS and VraR will be analyzed using immunoprecipitation and bacterial two hybrid system. We will structurally characterize these interactions by co-crystallizing the relevant proteins in order to understand the mechanism of the "Cross-talk" between the two two-component systems. Additionally, to check whether VraS can also activate the GraSR system, the genes under the GraSR regulation will be studied using qRT PCR in presence and absence of glycopepetide antibiotic stress, in a graS deletion mutant.

Antibiotic resistance in *S. aureus* is one of the leading causes of mortality and healthcare expenditure. Thus understanding the complex regulatory networks mediating such resistance is very important. This work will aid in deciphering the putative network that is responsible for the increase in glycopeptide tolerance in Sta.

Future Plans

In the future, we aim to enhance the arsenal of tools utilized to elucidate the functional mechanisms employed by transcription regulators. The molecular mass of the RNAP:TF:promoter complexes is generally greater than 600 KDa and hence it is possible to employ Cryo-Electron Microscopy (cEM) and small angle X-ray scattering

(SAXS) to interrogate these molecular assemblies. Initially, the complexes of regulatory factors with different RNAP subunits and cognate DNA sequences will be determined by macromolecular crystallography. This information will enable structure determination of RNAP:TF:promoter complexes using cEM and SAXS. We aim to employ this hybrid approach to study the structural mechanism of transcription regulation in the context of the RNAP holoenzyme and the complete genetic promoter.

In addition, we aim to develop methods that will allow validation of structural mechanisms derived in the appropriate organism. We will design and employ in vivo assays that in conjunction with aforementioned structural and biochemical tools- will provide a deeper and more fundamental understanding of the molecular basis of gene regulation.

Engineering of Nanomaterials for Biomedical Applications

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Research Theme

In our laboratory, we are using an an interdisciplinary approach using synthetic chemistry, cell biology and nanotechnology to address challenges in the area of membrane biohysics, cancer biology and infectious diseases; and developing nanomaterials for drug delivery, gene therapy and combination therapy.

Objectives

- Unlocking the Membrane Properties of Bile Acid Amphiphiles
- Engineering of Bile Acid Amphiphiles for Selective Antimicrobial Activity
- Controlled Drug Release based Hydrogels for Cancer Therapy

Progress of work during the current reporting year 2013-14

Scientific Reports

Unlocking the Membrane Properties of Bile Acid Amphiphiles

Cellular functions of mammalian cells depend on structure, composition, and dynamics of celular membranes,that also help in protection, control of transport of ions, nutrients, and serve as target of many drugs. Phospholipids, sphingolipids, and sterols build up the matrix of cellular membranes. Composition of cellular membranes and its interactions with external stimuli is critical for cell growth, differentiation, development, and cellular

movement. Phospholipids play critical role in structural and functional dynamics of cell membranes. Structural diversity within phospholipids assists in functional differences like lipid-signaling cascade, protective barrier at cell surface, transport of small molecules, and diffusion of integral proteins. Structural diversities in phospholipids is also crucial in evolutionary aspects of organisms as gram-negative bacterial membranes possess archaebacterial phospholipids and glycophospholipid Lipid A. Apart from these structural and functional differences, phospholipid-membrane (lipid-lipid) interactions are crucial for bacterial and viral infections, vesicular fusion like endocytosis and exocytosis, transport of cellular signals, import and export of cellular materials, and cellular signaling. Phospholipids based vesicular membranes also have the capability of drug encapsulation, and their controlled release at diseased sites. The interaction of these phospholipids with cellular membranes is critical for efficient transport of drugs in intracellular compartments.

Cationic amphiphiles on the other hand have been explored as delivery agents of genetic material to eukaryotic cells, drug encapsulation and delivery, and as antitumor, antibacterial, and antiviral agents. The bioactivity of these cationic lipids is strongly contingent on nature of the head group and hydrophobicity of lipids. Studies of interactions of cationic lipids with membranes are important to unravel the activity and mechanistic insights of these amphiphiles.

Therefore design of new steroidal phospholipids and cationic amphphiphiles, their interactions with membranes, and drug delivery propensities is key for efficient drug delivery vehicles.

Bile acids are principle constituents of bile apart from cholesterol, phospholipids and bilirubin. They are cholesterol-derived amphiphile molecules that help in absorption of fats and fat-soluble vitamins. Primary bile acids (Chenodeoxycholic acid, Cholic acid) synthesized in liver, get secreted from liver and get re-circulated via portal system. De-hydroxylation of primary bile acids by colon microorganisms converts them to secondary bile acids (Lithocholic acid, Deoxycholic acid). Bile acids present interesting materials for biomedical applications due to a) their facial amphiphilic, b) high biocompatibility, c) existence of diverse chemical scaffolds, and d) presence of reactive functional groups. Bile salts at sub-miceller concentrations are known to induce hydration of model membranes. Engineering and interactions of bile acid based materials with biomembranes would be critical for design of new nanomaterials for biomedical applications. Therefore, we

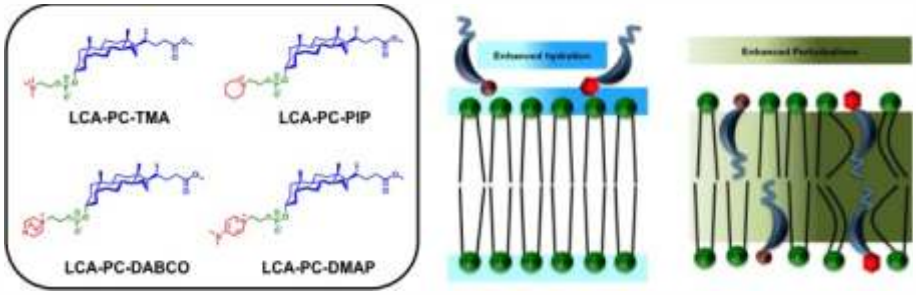

Figure 1. Lithocholic acid (LCA) derived phospholipids possessing unnatural head groups, and their interactions with model membranes showing that bulky head group induce more hydration and perturbations in model membranes

designed and engineered various bile acid amphiphiles and explored their properties of self-assembly, membrane properties, and their interactions with model membranes.

Phospholipid-membrane Interactions: Head group chemistry of phospholipids plays a critical role in fusion of drug delivery carriers with target cell membranes. We varied different polar head groups on lithocholic acid phospholipids from small hydrophilic to large hydrophobic head groups, and studied their interactions with model membranes. We studied the fusogenic and membrane properties of phospholipids possessing unnatural charged head groups with model membranes using Laurdan-based membrane hydration studies, DPH-based membrane fluidity, and differential scanning calorimetry. We unravel that fusogenicity, membrane hydration, and fluidity of membranes is strongly contingent on nature of the phospholipid charged head group. Our studies unraveled that introduction of bulky head groups like dimethylamino pyridine induces maximum membrane hydration and perturbations with high fusogenicity as compared to small head group based phospholipids (Figure 1). These phospholipids have also the capability of high retention in DPPC membranes. Hydration and fluidity of these phospholipid-doped DPPC membranes are contingent on nature of the charged head group. This study would help in future design of phospholipid-based nanomaterials for effective drug delivery.

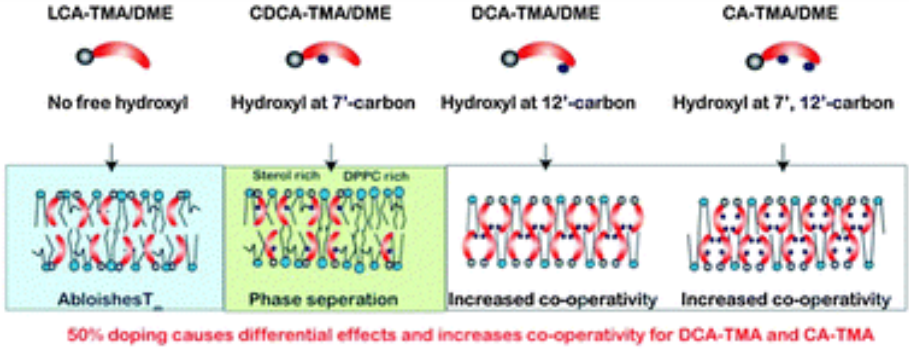

Figure 2. Schematic presentation of assembly of bile acid based cationic amphiphiles in model membranes showing the self-assembly properties of deoxycholic acid and cholic acid based amphiphiles.

Engineering of Bile Acid Amphiphiles for Selective Antimicrobial Activity

Tuberculosis (TB) is one of the most threatening communicable diseases caused by Mycobacterium tuberculosis. Currently used antimicrobial therapy involves the use of antibiotics that can target major biosynthetic pathways occurring in bacteria like biosynthesis of DNA, RNA, Proteins, peptidoglycans and folic acid. Targeting of these major pathways usually fail in eradicating the persistent infections involving slow-growing or non-growing bacteria. Presence of bacterial sub-populations having varying antibiotic susceptibility and inhabiting in macrophages further challenges the overall efficacy of antibiotics. Recently antimicrobials targeting the membrane organization of bacterial membrane or functions of membrane-associated enzymes have been proposed as effective therapeutics for targeting these persistent and slow-growing infections. As existing antimicrobial targeting the bacterial membrane organization is based on electrostatic interactions between these antimicrobials and negatively charged bacterial

membrane, it faces major drawback of poor selectivity against tuberculosis bacteria over other commensal bacteria. Their poor selectivity over mammalian cells also face challenges in their targeting bacteria residing in macrophages. Major challenges in design of new amphiphiles against bacteria are 1) their selectivity against particular bacteria, especially mycobacteria; 2) their targeting at the specific sites especially inside the macrophages in case of tuberculosis. We address the challenge of poor specificity of membrane disrupting antimicrobial amphiphiles against bacterial species, and present the discovery of high selective amphiphiles that are specific for tuberculosis responsible mycobacteria. These non-hemolytic amphiphiles are not active against other gram-positive and gram-negative bacterial species, and also non-toxic against lung epithelial cells and macrophages. In-depth mechanistic studies revealed that molecular differences in cell membrane of mycobacteria and other gram-positive/negative bacteria and their interactions with amphiphiles could be tuned to achieve the selectivity against mycobacteria. We showed that fine-tuning of charged head group modulates the specificity of amphiphiles against bacterial membranes. Hard-charged amphiphiles interact with mycobacterial trehalose dimycolates and penetrate through rigid mycobacterial membranes. In contrast, soft-charged amphiphiles specifically inhibit growth of both E. coli and S. aureus via electrostatic interactions. These subtle variations between interactions of amphiphiles and bacterial membranes could be explored further

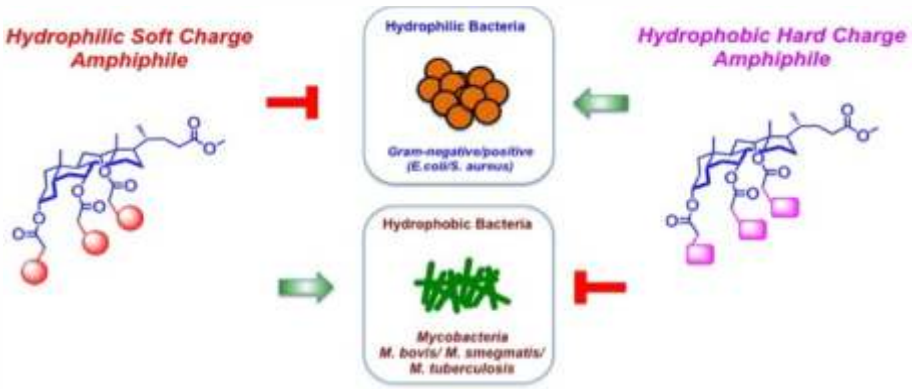

Figure 3. Schematic presentation showing the selective antimicrobial activity of hard-charged amphiphiles for mycobacteria, whereas soft-charged amphiphiles are selective for Gram-positive and gram-negative bacteria.

Hydrogels for Controlled Drug Release for Cancer Therapy

The efficacy of cancer chemotherapy is limited by systemic and cellular transport mechanisms of our body as most of drugs are administered orally or via intravenous routes. Administration of chemotherapeutics faces multiple challenges before they can act at the site of disease. Many pharmaceutics fall short of realizing their potential due to their poor bioavailability, short plasma half-life, low permeability across cell membrane, and rapid clearance from the body. To overcome these impediments, advanced drug delivery systems based on liposomes, polymers, and gold nanoparticles have been engineered and explored for drug delivery. These delivery systems are often non-discriminating, and deliver anticancer drugs to healthy tissues with detrimental effects. Therefore, there is an urgent need for localized drug delivery vehicles that would have beneficial characteristics of improved efficacy, reduced toxicity, and sustained drug release with minimal side effects.

Preliminary results indicate that TLE1 levels peak on days 3 and 4 of differentiation, while TLE2 levels show a graded increase with differentiation, as compared to the control GAPDH. We are expanding this study to include TLE3 and TLE4 also.

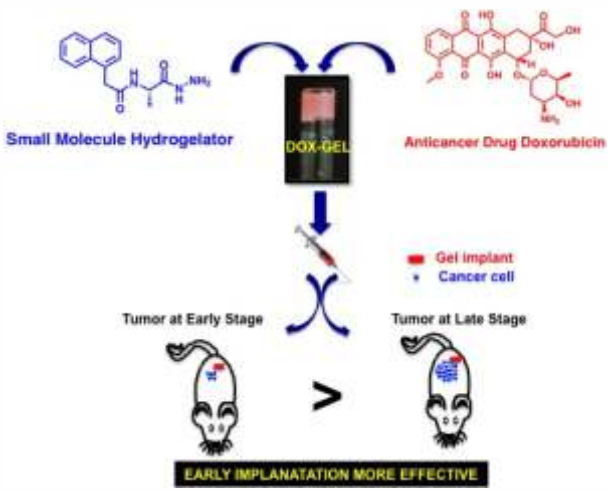

Figure 4. Schematic presentation of L-alanine based hydrogelator and encapsulation of doxorubicin in this gel, showing injectability and in vivo tumor regression at early stages of cancer development.

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

Localized drug delivery involves the use of implantable or injectable systems with sustained drug release characteristics that would prevent the growth of cancerous cells which cannot be removed during resection. Self-assembling drug delivery systems open up new avenues for chemists to design efficient delivery agents whose release-profiles could be altered by chemical and/or physical stimuli. LMHGs have specific advantages over their polymeric counterparts for localized drug delivery like lower cytotoxicity, lesser immunogenicity, predictable degradation pathways, and formation of networks like ECM. However, since the intermolecular forces holding these molecular assemblies are often weak, they readily fall apart in presence of the shear forces encountered during drug encapsulation or injection through syringe. To best of our knowledge, injectable drug delivery systems using LMHGs (M.W. <300Da) for in vivo applications have not been explored. We have shown low molecular weight derivatives of L-alanine as a new paradigm in development of injectable localized drug delivery agents. These derivatives undergo self-assembly in aqueous medium through inter-molecular hydrogen bonding, and can readily entrap DOX, and still form injectable assemblies (Figure 4). We noticed that DOX is covalently attached with gelator through imine bond, which is known to be unstable under acidic conditions found in vicinity of tumors. The DOX-containing gels were able to regress tumor load at palpable stage when injected at site of tumors. We believe that slow release of DOX caused higher reduction in tumor burden in tumor models at their early stages.

Scientific Reports

Future plans

There has not been enough progress in literature on design of new phospholipids for drug delivery in cancer. To our knowledge there is no report on synthesis and anticancer

activities of phospholipid-drug conjugates. Existing and reported liposomal formulations for cancer therapy suffer from limitations such as the potential for leakage and release of the active drug is not directly coupled to the mechanism activating the carrier. We propose to design and synthesize steroid moiety based phospholipids and its drug conjugates, studying their membrane properties, drug encapsulation, drug release and anticancer activities. We would like to learn the consequences on bilayer properties of amphiphilic bile acid derivatives that would be unable to transfer between membranes. We will synthesize lithocholic acid based phospholipid-drug conjugates for drug delivery applications. We hypothesize that design of new phospholipids, and phospholipid-drug conjugates would provide us better platforms for better drug delivery systems for cancer therapy.

Presence of bacterial sub-populations having varying antibiotic susceptibility and emergence of multi-drug resistance (MDR) by these bacteria challenges the overall efficacy of antibiotics. Therefore, targeting of these major pathways usually fail in eradicating the multi-drug resistance infections, persistent infections, and biofilms involving slow-growing or non-growing bacteria. Recently antimicrobials targeting the membrane organization of bacterial membrane or functions of membrane-associated enzymes have been proposed as effective therapeutics for targeting these persistent, slow-growing and MDR infections. We plan to engineer amphiphiles and amphiphile-antibiotic conjugates to achieve synergistic effects against multi-drug resistant, persistent infections and against biofilms. Various antibiotics are known to alter the essential functions of bacteria that however are not active against MDR, persistent infections and biofilms. In contrast amphiphiles mimicking AMPs can selectively disrupt the barrier function of bacterial membranes and can translocate across membranes. Therefore we aim to engineer hybrid amphiphile-antibiotic conjugates to combat these persistent infections.

Publications

Original peer-reviewed articles

1. Bansal S, Singh M, Kidwai S, Bhargava P, Singh A, Sreekanth V, Kundu S, Singh R and Bajaj A (2014) Bile Acid Amphiphiles with Tunable Head Groups as Highly Selective Non-hemolytic Antitubercular Agents. ***Med Chem Commun*** (in Press)
2. Singh M, Kundu S, Reddy A, Sreekanth V, Motiani RK, Sengupta S, Srivastava A and Bajaj A (2014) Injectable Small Molecule Hydrogel For Localized and Sustained In Vivo Delivery of Doxorubicin. ***Nanoscale*** (In Press)
3. Singh K, Verma V, Yadav K, Sreekanth V, Kumar D, Bajaj A, Kumar V (2014) Design, regioselective synthesis and cytotoxic evaluation of 2-aminoimidazole-quinoline hybrids against cancer and primary endothelial cells. ***Eur J Med Chem*** 87C:150.
4. Singh M and Bajaj A (2014) Unraveling the Impact of Hydroxylation on Interactions of Bile Acid Cationic Lipids with Model Membranes by In-depth Calorimetry Studies. ***Phys Chem Chem Phys*** 16: 19266.
5. Bhargava P, Singh M, Sreekanth V and Bajaj A (2014) Nature of the Charged Head Group Determines the Fusogenic Potential and Membrane Properties of Lithocholic Acid Phospholipids. ***J Phys Chem B*** 118:9341.

Modulation of Host Immunity and Nutrient Allocation by Biotrophic Pathogens

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Research Theme

Obligate biotrophic pathogens like the powdery mildew fungi manipulate living plant hosts to acquire nutrients while limiting host defense responses. We aim to elucidate the molecular mechanism(s) mediating these interactions via infection site-specific analyses, with the long-term goal of engineering durable powdery mildew resistance in food legumes.

Objectives

The major goal of our research is to identify and target novel host genes associated with defense that limit growth of the pathogen as well as host compatibility factors required for the growth and reproduction of the pathogen. We envisage that targeting a combination of such factors would result in dramatically reduced pathogen proliferation and contribute to durable resistance that is less likely to be rapidly overcome by pathogen counter-evolution. The following major objectives will be pursued:

- Identification of novel infection site-specific host factors controlling powdery mildew proliferation using a model pathosystem
- Elucidation of factors mediating carbon (re)allocation at the host-pathogen interface
- Translation of selected targets into food legumes of agronomic import

Future Plans

Powdery mildew is one of the most devastating fungal pathogens limiting productivity of food legumes in India and other developing countries. It can reduce pea and mung bean yields by 25-50% and is emerging as a significant pathogen on chickpea. Food legumes are an important source of dietary protein, essential amino acids, and fiber and on average contribute 10-20% of total protein intake in developing countries. Legumes also play a pivotal role in maintaining our ecosystem by fixing atmospheric nitrogen in symbiotic association with soil rhizobial bacteria, enabling sustainable farming practices and reduced fertilizer use.

Powdery mildew fungi are obligate biotrophic pathogens that can only propagate on living plant cells. They grow superficially on leaves, stems, and sometimes fruits and appear as a fine white powder on the host surface. During pathogenesis (Figure 1), they siphon nutrients from the plant host through haustoria formed within host epidermal cells while

suppressing defense responses. The fungi accomplish this by targeting host genes and/or proteins, known as compatibility factors, specifically at the interaction site. Wind-dispersed asexual conidiospores rapidly propagate new infections. Current chemical methods used to control the disease are neither economical nor environmentally sustainable. Furthermore, few powdery mildew resistant legume varieties have been identified, with the identity of the genes conferring resistance and knowledge of the underlying molecular mechanisms not fully understood. Therefore, there is a need to develop novel strategies to combat this disease. Our research will contribute to this endeavor by combining functional genomics and molecular genetics to elucidate the molecular mechanisms underlying the powdery mildew-legume interaction and identify novel genes that limit powdery mildew proliferation.

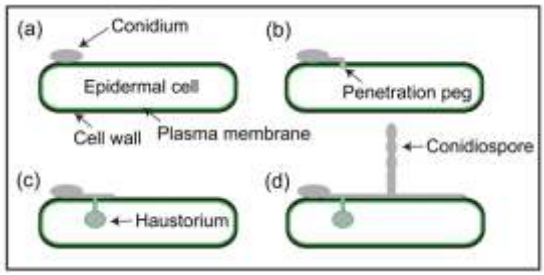

Figure 1. Schematic of powdery mildew infection process

The *Medicago truncatula-Erysiphe pisi* pathosystem:

The compatible *Medicago truncatula-Erysiphe pisi* interaction will be used as a model to identify novel host factors that limit powdery mildew proliferation on legumes. Key attributes that make the legume *M. truncatula* an excellent model are its relatively small diploid genome (~400 Mbp), short generation time, prolific seed production, high degree of synteny and co-linearity with agriculturally important legumes, availability of mutant populations for forward and reverse genetics and genomics tools including the completed genome sequence, and ease of transformation. In addition, availability of powdery mildew resistant and susceptible *M. truncatula* accessions will help uncover novel host defense genes and compatibility factors vital for the survival of the pathogen. The powdery mildew pathogen *E. pisi's* newly completed genome sequence and the ability to infect both *M. truncatula* and important food legumes makes it a valuable model to study legume-pathogen interactions. Importantly, information gleaned from this model pathosystem can be readily translated to agriculturally important food legumes.

Identification of novel plant factors controlling powdery mildew proliferation in legumes:

The structural development and progression of powdery mildew infection on the host plant is well defined and highly localized. Hence, it is highly amenable to site-specific profiling, which can dramatically increase sensitivity and uncover specific host processes and process components hidden in whole-leaf global expression analyses. Laser microdissection (Figure 2) will be employed in combination with next generation sequencing to specifically profile the transcriptome of plant cells at the infection site at various time points after pathogen inoculation in *M. truncatula* accessions with altered powdery mildew resistance. An integrated Bioinformatics approach will be utilized to identify host components, processes and regulators with infection site-specific expression followed by functional characterization of selected candidates using reverse genetics.

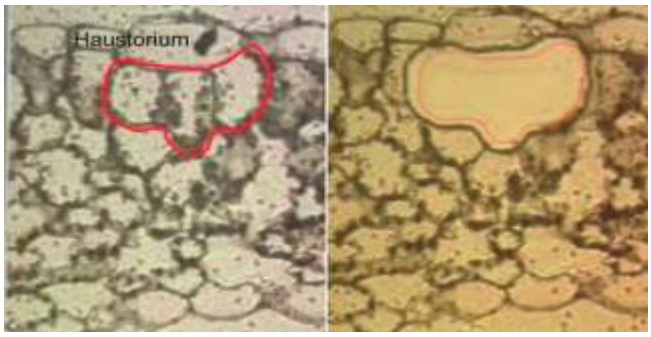

Figure 2. Example of powdery mildew infection site-specific isolation of plant cells using laser microdissection

Appropriate *M. truncatula* mutants and/or over-expression lines will be obtained or generated and assayed for altered pathogen resistance phenotypes. Wherever necessary, double or triple mutants will be tested to address functional redundancy. Candidates with a clear powdery mildew phenotype that have no impact on growth or on plant defense hormone signaling pathways will be prioritized. It is well known that increasing levels of the plant defense hormone salicylic acid can not only impact growth but also levels of other hormones (e.g. Ethylene/Jasmonic acid), which, for instance, could make plants more susceptible to necrotrophs. Using this strategy, I expect to uncover novel host genes associated with defense that limit growth of the pathogen as well as host compatibility factors required for growth and reproduction of the pathogen.

Elucidation of factors mediating carbon (re) allocation at the powdery mildew infection site:

Biotrophic pathogens like the powdery mildew fungi acquire nutrients, especially sugars, from their host and consequently trigger a source-to-sink transition in infected host tissues. Although glucose appears to be the major carbon that is imported from the host and is essential for growth and metabolism of the fungus, sucrose or fructose may also be transported.

It has been proposed that hexose sugars are made available at the plant-microbe interface through the action of plant and fungal cell wall invertases (Figure 3). These sugars can then be preferentially taken up by the plant or the microbe through specific transporters. Alternatively, sugars may also accumulate at this interface via plant SWEET transporters, which have recently been shown to be induced by specific effectors released by some bacteria. Since this alteration in the source-sink relationship at the powdery mildew infection site is critical to the growth of the pathogen and few molecular components are known, we intend to identify key players and regulators mediating this response.

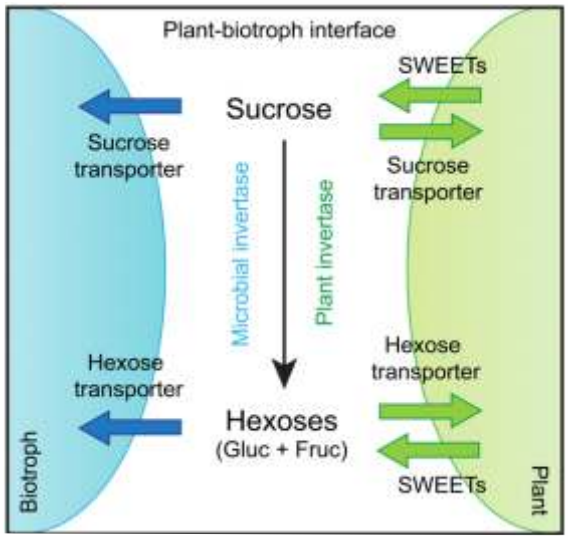

Figure 3. Proposed model for carbon (re) allocation at the plant-biotroph interface

Initially, publicly available *M.*

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truncatula gene expression databases will be mined for candidates based on locale (e.g. cell/tissue type) and context (e.g. during source-sink transition, biotic stress, etc.) of expression. In parallel, *M. truncatula* homologs of *Arabidopsis* genes previously shown to be induced in an infection site-specific manner (e.g. cell wall invertase βFRUCT1 and glucose transporter STP4), will be identified. Quantitative PCR and promoter::reporter assays will be performed to confirm the inducible and site-specific pattern of expression of selected candidates. Functional relevance will be tested using infection site-specific knockdown or over-expression lines in *M. truncatula* and/or *Arabidopsis*, as needed. We anticipate that altering the expression of genes essential for nutrient acquisition specifically at the site of infection will limit sugar availability and consequently inhibit pathogen growth. To identify key regulators of this process, we will combine our infection site-specific profiling data (above) with promoter, co-expression and gene ontology enrichment analyses to construct putative regulatory networks, which can then be experimentally verified. Using these approaches, we expect to identify important players modulating carbon (re)allocation at the powdery mildew infection site, which could be shared by other biotrophs.

Translation of selected targets into food legumes of agronomic import:

Several food legumes are currently the target of extensive sequencing-based genomics research. Therefore, a long-term goal is to translate our basic findings in M. truncatula to relevant crop legumes, with relevance determined based on several criteria, including disease severity, availability of genomics/genetic resources and ease of transformation.

Mechanisms of Cell Division and Cellular Dynamics

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Research Theme

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

Objectives

We wish to uncover the molecular mechanisms of mitotic regulation by the Light Intermediate Chain (LIC) subunits of cytoplasmic dynein. In other projects, we wish to uncover the role of the exocytic membrane trafficking machinery during cytokinesis, the physical separation of daughter cells at the end of mitosis. Our long-term interests include elucidating the mechanistic bases for biogenesis and function of novel modes of intercellular communication.

Progress of work during the current reporting year 2013-14

Cytoplasmic dynein performs a plethora of functions during mitosis. Our quest to understand the mechanistic roles performed by the LIC subunits of dynein during mitosis led us to uncover novel pleiotropic functions for LIC2 during metaphase. We had reported last year that LIC2 depletion in Hela cells surprisingly showed us a metaphase arrest as potent as for LIC1 depletion, but mechanistically independent of it. We showed that LIC2 acts through/ on an active SAC to mediate mitotic progression. LIC2 depleted metaphase cells showed higher average inter-kinetochore distances in metaphase cells as compared to control cells, suggesting that LIC2 depletion leads to inefficient inactivation of the SAC. These results pointed to a direct role for LIC2-dynein in removal of checkpoint proteins from metaphase kinetochores.

We performed quantitative immunofluorescence of various SAC proteins at metaphase kinetochores in normal and LIC2 depleted cells to check whether LIC2 depletion led to the accumulation of SAC proteins on metaphase kinetochores. Cells treated with either control

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or LIC2 siRNA for 48 hours were observed by time lapse imaging on gridded coverslips, and cells that arrested in metaphase for greater than 60 minutes (indicating efficient LIC2 knockdown) were identified. Following immunostaining of kinetochores, SAC protein (Mad1/ Mad2/ BubR1 in independent experiments) and chromosomes on the same cover slips, we quantified the amounts of individual SAC proteins present on metaphase kinetochores. We found that all three SAC proteins, Mad1, Mad2 and BubR1 fail to get removed efficiently from metaphase kinetochores upon LIC2 depletion, strongly implicating LIC2 in SAC silencing at metaphase (Figure 1). Significantly, this is among the first reports of cytoplasmic dynein playing a direct role in silencing both the microtubule attachment and inter-kinetochore tension sensing stages of the SAC. In this aspect, it appears that LIC2 plays a broader role in dynein mediated SAC silencing than LIC1, which removes only the attachment sensing SAC proteins (Figure 1,1).

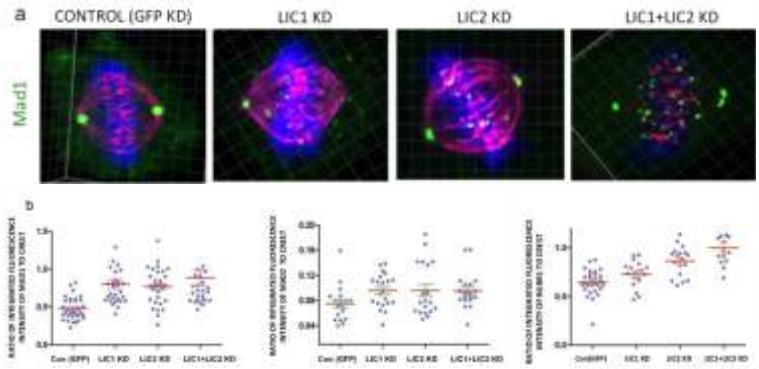

Figure 1. LIC2 depleted cells accumulate both attachment and tension sensing SAC proteins at metaphase kinetochores. Hela cells were treated with control or LIC2 siRNA for 48 hours and imaged live on gridded cover slips to identify cells arrested in metaphase for longer than 60 minutes (indicating LIC2 knockdown). Following immunostaining with antibodies against CREST (red, kinetochores), SAC protein (green), microtubules (magenta) and DAPI (chromatin), the arrested cells were imaged using confocal microscopy. a) Representative images showing accumulation of the SAC protein Mad1 at metaphase kinetochores upon either LIC2 or LIC1 depletion, as compared to control cells b) Quantification of the fluorescence intensity of 3 different SAC proteins - Mad1, Mad2 and BubR1 - at metaphase kinetochores. Y-axis depicts the mean fluorescence intensity (+/- SD) normalized to the respective kinetochore (CREST) intensity for all kinetochores in the cell. n = 3 independent experiments. All kinetochore pairs for ~ 15 metaphase cells per experiment were measured.

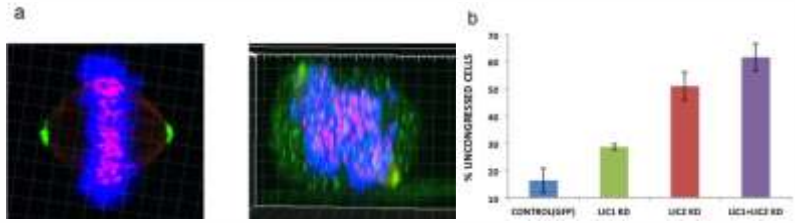

Figure 2. LIC2 depletion leads to chromosome congression defects in metaphase. a) Hela cells were treated with control (left) or LIC2 (right) siRNA for 48 hours, suitably immunostained and imaged. a) Representative images showing drastic chromosome congression defect upon LIC2 depletion, but not significantly upon LIC1 depletion, as compared to control cells b) Quantification of the chromosome congression defects. Y-axis depicts the fraction of cells with uncongressed chromosomes (mean +/- SD). n = 3 independent experiments, ~ 100 metaphase cells per experiment.

Cytoplasmic dynein facilitates chromosome congression to the metaphase plate. We found that LIC2 played a major role in ensuring proper chromosome congression as opposed to LIC1, which plays only a minor role in this function (Figure 2).

The above roles of LIC2 in SAC silencing and chromosome congression pertain to chromosome and kinetochore related functions. We next probed whether LIC2 is responsible for other mitotic functions of dynein, especially those involved in spindle assembly and organization. A striking observation was that mitotic spindles were significantly elongated in LIC2 depleted cells (Figure 3a), with the spindle poles almost juxtaposed with the cell cortex (not shown). This effect was much less pronounced for LIC1 depletion (Figure 3a). A related and equally prominent observation was the shortening of astral microtubules that connect the spindle poles to the cell cortex, which was also highly pronounced upon LIC2 depletion but not upon LIC1 depletion (Figure 3b). These observations suggested a strong involvement for LIC2 in regulating the size of the mitotic spindle by maintaining balanced opposing forces with kinesin motors during spindle assembly and maintenance.

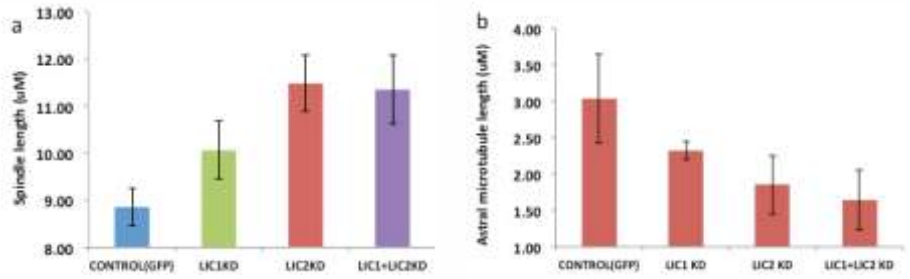

Figure 3. LIC2 regulates mitotic spindle length and astral microtubule length in metaphase. Hela cells were treated with control or test siRNA for 48 hours, suitably immunostained and imaged in metaphase. a) The total length of the mitotic spindle is significantly increased upon LIC2 depletion b) The length of astral microtubules nucleated from the poles is significantly reduced upon LIC2 depletion. Error bars are mean +/- SD from 3 independent experiments, ~ 30 metaphase cells per experiment.

An important implication of the loss/ reduction in length of astral microtubules is that the centrosome/ microtubule organizing centres (MTOCs) are severely compromised in their ability to nucleate microtubules in the absence of LIC2. Astral microtubules are known to play critical roles in anchoring the spindle pole to the cell cortex through the action of cortical dynein, to position the entire mitotic spindle properly with respect to the cell body and the substratum. Defective anchoring of spindle poles leads to mis-oriented spindles, which ultimately govern the plane of cell division. The orientation of cell division plays key roles in stem cell self-renewal vs. differentiation decisions; spindle mis-orientation is suggested as a very likely cause for disorders like polycystic kidney disease. LIC2 depletion led to large spindle axis angles with respect to the substratum as compared to control cells, whereas LIC1 depletion showed only a mild defect (Figure 4). These observations suggested a major role for LIC2-dynein in ensuring proper orientation of the mitotic spindle. It was observed that LIC2 also plays a mild role in spindle pole focusing in mammalian cells as opposed to a stronger role shown by LIC1 in ensuring proper spindle pole focussing at the MTOCs (data not shown). The overlapping but varying magnitudes of different mitotic functions played by LIC1 and LIC2 suggest a division of labour with respect to mitotic functions between the two independent dynein complexes containing LIC1 or LIC2.

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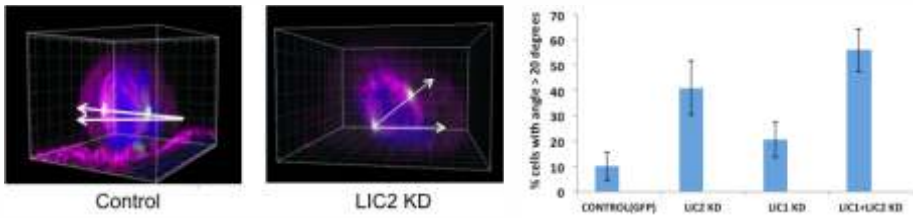

Figure 4. LIC2 regulates mitotic spindle orientation. Hela cells were treated with control or test siRNA for 48 hours, suitably immunostained and imaged in metaphase using confocal microscopy. The angle made by the axis passing through the mitotic spindle poles with the substratum (cover slip) was measured. Left: Spindle angle in representative control and LIC2 depleted Hela cells depicted from 3D reconstructions of confocal z-stacks. Right: Y-axis represents the fraction of metaphase cells with a spindle angle (tilt) of > 20 degrees. Error bars are mean +/- SD from 3 independent experiments, ~ 30 metaphase cells per experiment.

We next aimed to define the molecular mechanism by which LIC2 regulates the various spindle related functions described above. The protein NUMA (Nuclear Mitotic Apparatus), a large conserved nuclear protein has been clearly implicated in regulating spindle pole nucleation ability as well as being a key player in controlling spindle pole anchoring to the cell cortex and in consequently regulating spindle orientation (2-5). NUMA leaves the confines of the nuclear membrane after nuclear envelope breakdown at the beginning of mitosis; it simultaneously starts populating the spindle poles (2), as does LIC2 (data not shown). It has also been reported that NUMA is transported by dynein along the mitotic spindle fibres to the poles as part of maturation the centrosome (6). LIC1 (but not LIC2) has been shown to transport the centrosomal protein pericentrin to spindle poles, however the

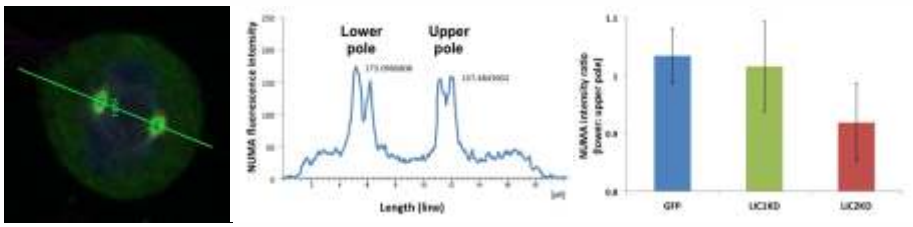

Figure 5. LIC2 is responsible for loading of NUMA on to spindle poles during mitosis. Hela cells were treated with control or test siRNA for 48 hours, immunostained and imaged in metaphase using confocal microscopy. Left: The fluorescence intensity in the NUMA channel was measured using linescans through a linear axis running through the spindle poles. Middle: The linescan showing the fluorescence intensity profile of a control cell along the pole axis. Right: LIC2 depletion significantly reduces the loading of NUMA preferentially at the upper pole. Y-axis represents the ratios of peak NUMA intensity for upper vs. lower poles across 14 - 22 metaphase cells per experiment. Error bars are mean +/- SD from 3 independent experiments.

subunit(s) of dynein required for transporting NUMA to poles have not been identified.

We found that LIC2 is required for transport of NUMA to spindle poles. NUMA normally organizes into ring-like structures around the centrosomes, a phenomenon that we could readily observe in control cells both visually as well as by quantifying fluorescence intensity linescans (Figure 5). Depletion of LIC2 led to quantifiable reduction in the amounts of NUMA immunofluorescence at the spindle poles. Notably, careful analysis of the linescans revealed that the reduction of NUMA intensities was seen preferentially at the "upper"

spindle pole (Figure 5). This result indicated that LIC2-dynein is responsible for transport of NUMA preferentially to one spindle pole (likely the daughter centrosome) and consequently prevents maturation of this centrosome, rendering it defective in nucleating astral microtubules.

To ascertain whether LIC2 developmentally regulates cell divisions *in vivo*, we probed the function of LIC2 in controlling early divisions in the zebrafish (*Danio rerio*) embryo. Briefly, control or LIC2-specific morpholinos were injected into the yolk of one-cell embryos from the same clutch. Microscopic observation of the embryos in multiple experiments showed that a significant number of them (about 30%, data not shown) developed a distinct, single "furrow" in the middle of the layer of epiblast cells around 3.3 hours after injection (Figure 6a), while water injected embryos grew normally. This stage corresponds to the 256-512 cell stage in control embryos preceding gastrulation. All of the furrowed embryos died within a few hours after injection. Metaphase cells of furrowed (LIC2 depleted) embryos showed significantly elongated spindles (Figure 6b,c). Additionally, metaphase cells of LIC2 depleted embryos showed dispersed spindle poles (Figure 6b), similar to the mild dispersion phenotype seen in mammalian cells. Significantly, LIC2 depleted embryos also showed less total number of cells at the same time after injection, suggesting that there is a proliferation defect upon LIC2 depletion. This result is likely due to defects in SAC silencing leading to metaphase arrest upon LIC2 knockdown seen in mammalian cells. These results indicate that LIC2 plays conserved roles in spindle organization and SAC silencing across metazoans, and is essential for governing early cell divisions in vertebrate embryos.

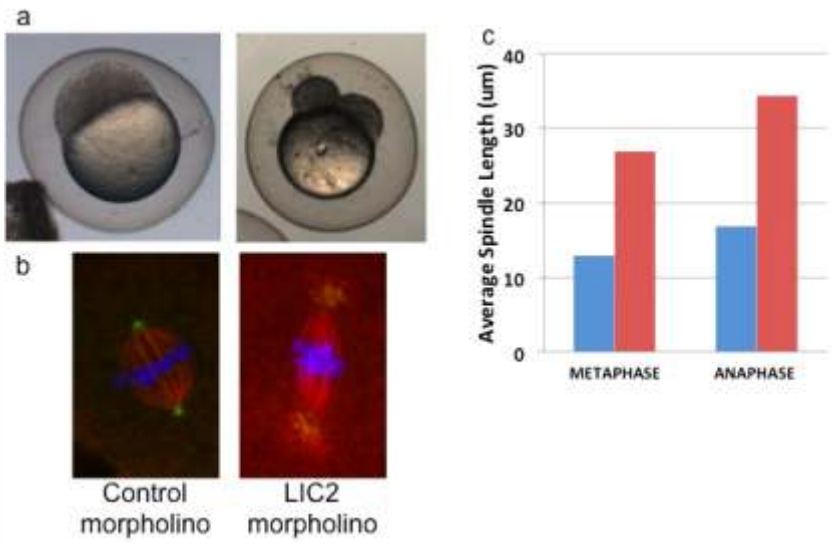

Figure 6. LIC2 is required for early zebrafish embryogenesis by regulating mitotic spindle length and spindle pole focusing. One-cell zebrafish embryos were injected with water or LIC2 morpholino. a) Injected embryos were visualized by a light microscope at equivalent times after injection (3.3 hours), when a single, prominent furrow in the cell mass was consistently observed. b) Embryos were fixed and immunostained for microtubules (red, alpha tubulin), spindle poles (green, gamma tubulin) and chromosomes (blue, DAPI), and imaged in metaphase using confocal microscopy across the cross section. c) Treatment with LIC2 specific morpholinos led to significantly longer spindles and poorly focused spindle poles in the furrowed embryos. Blue bars = control cells, red bars = LIC2 depleted cells.

Scientific Reports

Based on these observations, we postulate a comprehensive model for the pleiotropic functions of LIC2 during metaphase (Figure 7). LIC2-dynein is responsible for congression of condensed chromosomes to the equatorial plate of the cell during metaphase. Following this, LIC2-dynein transports NUMA to spindle poles. LIC2-dynein also transports SAC proteins, specifically Mad1, Mad2 (attachment sensors) and BubR1 (tension sensor) away from kinetochores, thus helping in inactivating the SAC. At spindle poles, LIC2-dynein also helps focus dispersed minus ends of microtubules to the centrosomes. Additionally, LIC2-dynein present at the cell cortex near the spindle poles helps to anchor at least one of the spindle poles, likely the younger daughter centrosome, to the cell cortex via its interaction with cortical NUMA, which can interact with the plasma membrane. Through this anchoring, LIC2 aids in maintaining proper mitotic spindle orientation and thus regulates the plane of cell division.

Future Plans

We are probing other mechanistic questions regarding the roles of LIC1 and LIC2 in mitosis. What are the mechanisms by which LIC1 and LIC2 phosphorylation regulate their mitotic functions? What are the mitotic interactomes of LIC1 and LIC2? What are the three-dimensional atomic resolution structures of LIC1 and LIC2? Finally, what are the detailed and distinct developmental consequences of perturbing the function of LICs in metazoan embryos? Answers to these questions will give us a holistic understanding of the molecular mechanisms by which dynein LICs regulate mitotic progression.

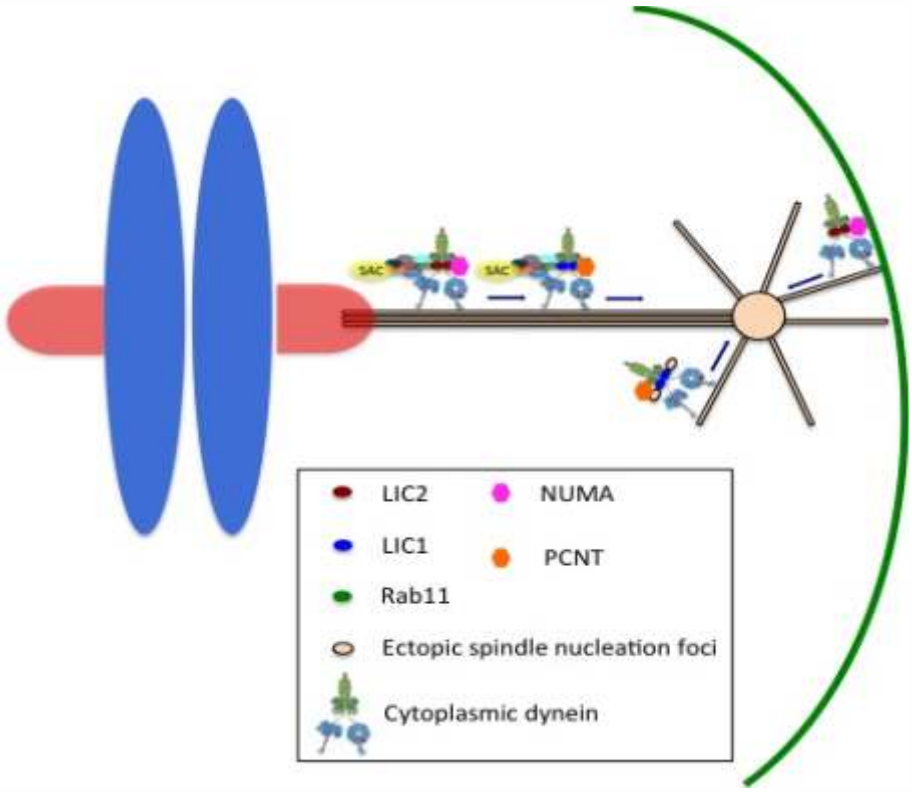

Figure 7. A comprehensive model for the pleiotropic functions of LIC2 during mitosis.

Effector-Triggered Immunity: Elucidating Inositol Regulated Pathways in Assemblies and Signaling by Immune Regulators

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Research Theme

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

Objectives

Our earlier work has identified that membrane localized complexes of R proteins (such as resistance to *Pseudomonas syringae* 4 or 6; RPS4 or RPS6) with regulators of immunity such as Suppressor of RPS4-RLD 1 (SRFR1) and Enhanced Disease Susceptibility 1 (EDS1) are disrupted by effectors such as AvrRps4 and HopA1 likely suggesting direct gateway towards ETI signaling. We are focused on the role of inositol derivatives in the assembly of defense proteins and signaling during ETI. Inositol phosphates (InsPs) define secondary messengers in most eukaryotic systems. Lipid-conjugated inositols, phosphatidylinositols (PtdIns), define membrane architectures. Our investigations involve the following approaches:

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

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Progress of work during the current reporting year 2013-14

In preliminary qPCR assays, we have identified that several InsP biosynthesis and metabolism genes are significantly reduced in ***srfr1-4***, a mutant with unregulated defenses (Fig. 1).

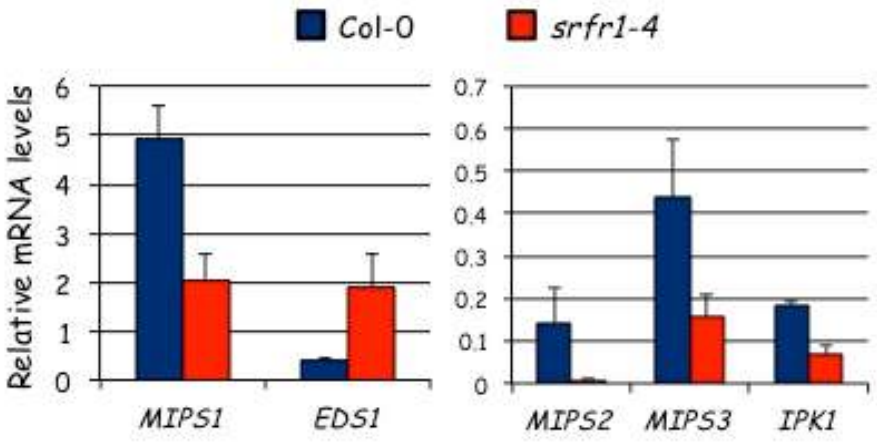

Figure 1. Transcript levels of myo-INOSITOL PHOSPHATE KINASE isoforms (MIPS1-3), EDS1 and IPK1 measured by qPCR in wild-type (Col-0) and srfr1-4 plants. The transcript levels were standardized using the SAND gene (At2g28390) trasncripts as internal standards.

The enhanced resistance displayed by the srfr1-4 plants may likely be a consequence of these altered levels of InsPs. Interestingly, SRFR1 is co-regulated with *INOSITOL PENTAKISPHOSPHATE KINASE 1 (IPK1)*, a enzyme involved in the biosynthesis of phytic acid (InsP6). We have obtained T-DNA tagged mutant plants of *IPK1 (ipk1-1)*. These plants are slightly stunted in appearance when grown under long day conditions and are reminiscent of similar growth phenotype of the *srfr1-4* plants. In preliminary assays *ipk1-1* is enhanced resistant to DC3000 suggesting that genetically IPK1 or InsP6 functions as a negative regulator of defense. Whether lower levels of InsP6 or accumulation of lower InsPs (such as InsP5, InsP4 and InsP3) in the *ipk1-1* accounts for the enhanced resistance is being investigated.

Phospholipases (PLCs) are activated in a biphasic manner during ETI. The byproducts, InsPs and phosphatidic acid functions as potent secondary messengers. Although several phosphatidic-acid binding proteins have been identified, their roles in plant defenses remain elusive. Equally the roles of InsPs remains to be explored. Time-dependent profiling of InsPs during ETI will be extremely important to elucidate functions of specific InsPs in immune responses. Plants with lower InsP3 have been reported to be enhanced susceptible to DC3000 whereas our initial results on *ipk1-1*, which likely has reduced levels of InsP6 (and likely accumulated levels of InsP3) are enhanced resistant, suggest that different InsPs contribute differentially to defense responses. We have initiated non-radioactive ion-exchange based quantification of InsPs. In parallel we are generating inducible transgenic expression lines of AvrRps4 which will provide us a system to induce and track ETI in a controlled time-depenedent manner.

Immune assemblies of RPS4, EDS1 and SRFR1 are anchored on membranes via unknown mechanisms since none of these proteins contain predicted transmembrane domains. Likely these associations on membranes are facilitated by binding to phosphatidylinositols (PtdIns). How the effectors AvrRps4 and HopA1 perturb these assemblies are not known. In a recent advancement the bacterial effector HopA1 was reported to bind PtdIns. We are expressing EDS1 and SRFR1 in heterologous systems to test for PtdIns binding in in vitro and in vivo assays.

Future Plans

- We will continue the further molecular characterization of *ipk1-1* and other inositol biosynthesis and metabolism mutants. This will include testing for altered defense responses, qPCRs for defense-related genes and generating combinatorial mutants with plants deficient in immune responses. This approach will be beneficial in identifying genetic links between InsPs and defense players.
- Of primary importance for our research is the standardization of chromatography based quantification of InsPs. Once optimized InsPs will be first compared among wild-type, *ipk1-1* and *srfr1-4*.
- Unlike animal systems, the receptor of InsP3 that facilitates Ca2+ release from intracellular stores have not been identified. Current understanding suggest that InsP3 in plants is converted to InsP6. We are initiating a collaboration with Dr. M.S. Shashidhar, Organic Chemistry Division, National Chemical Laboratory, Pune, India to synthesize matrix-conjugated and labeled InsP6 in order to identify InsP6-binding proteins in plants.
- In an ongoing collaboration with Dr. Walter Gassmann, University of Missouri-Columbia, USA we are investigating the role of SUMOylation in EDS1 function. In recent observations, eds1 containing a lysine to arginine substitution (K478R) in the predicted SUMOylation motif is non-functional in immunity. We are currently optimizing *in vitro* and *in vivo* SUMOylation detection assays to test for SUMOylation of EDS1.

Publications

Original peer-reviewed articles

1. Kim SH, Son GH, Bhattacharjee S, Kim HJ, Nam JC, Nguyen PD, Hong JC and Gassmann W (2014) The Arabidopsis immune adaptor SRFR1 interacts with TCP transcription factors that redundantly contribute to effector-triggered immunity. ***Plant J.*** 78: 978.

Reviews/Proceedings

2. Bhattacharjee S, Garner CM and Gassmann W (2013) New clues in the nucleus: Transcriptional reprogramming in effector-triggered immunity. ***Frontiers Plant Sci.*** 4: 364.

Scientific Reports

Complement Regulatory Proteins in Maintenance of Immune Tolerance

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Research Theme

Complement regulatory proteins enable complement activation on pathogenic surfaces while protecting self-cells from damage. Their absence often predisposes individuals to autoimmunity. In this context we propose to understand immunological circuits that malfunction in the absence of a complement regulator of the alternate pathway called complement factor H related protein1 (CFHR1).

Objectives

- Assessing proportion and function of immune cells derived from peripheral blood of healthy asymptomatic individuals lacking *CFHR1* gene.
- Understanding the role of *CFHR1* gene in breakdown of B cell tolerance in pediatric samples from anti-complement factor H (CFH) -positive individuals lacking kidney damage and comparing it to anti-CFH autoantibody positive samples with kidney pathology.

Progress of work during the current reporting year 2013-2014

It has been known for long that complement system plays a central role in inflammatory responses and tissue/organ injury that follows direct immune recognition by antibodies as well as in processes such as, organ ischemia/reperfusion. Therefore fine-tuning of the complement system becomes extremely important to restrict tissue damage and prevent autoimmunity. Within the complement system, three pathways are capable of initiating the processes that result in C3 activation: classical, alternative and lectin. In contrast to the specific target-protein/carbohydrate and recognition protein interaction that characterize the other two pathways, the alternate pathway is capable of autoactivation because of "tickover of C3" or the spontaneous hydrolysis of C3. This necessitates continuous active control of this pathway in the form of various membrane-bound or fluid-phase regulators. Any aberration in the control due to mutations or absence of regulatory proteins therefore predisposes an individual to autoimmunity, a classic example of which is the atypical hemolytic uremic syndrome (aHUS). In most patients with aHUS, it has been demonstrated that chronic, uncontrolled, and excessive activation of complement can result from production of anti-factor H autoantibodies or from genetic mutations in any of several complement regulatory proteins (e.g., factor H, factor H related protein1 (CFHR1) or CFHR3, membrane cofactor protein, factor I, factor B, complement C3, and

thrombomodulin; Figure 1). This result in platelet activation, damage to endothelial cells, and white blood cell activation, leading to systemic thrombotic microangiopathy, which manifests as decreased platelet count, hemolysis, damage to multiple organs, and often, death.

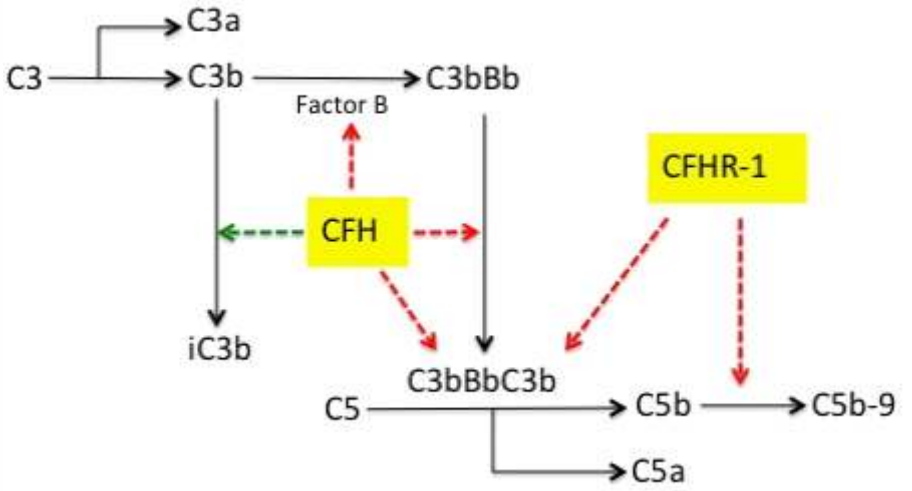

Figure 1. CFH and CFHR mediated regulation of alternate pathway of complement activation

A multicentric report from India describes that 50-60% aHUS patients have circulating anti-CFH antibodies. This is in wide contrast to available literature that indicates 5-25% patient cohorts from Europe having the autoantibody. But in conformity with European cohorts, 90% of the autoantibody containing patients in northern India had homozygous deletions in *CFHR1* gene. Hence, the absence of *CFHR1* gene clearly predisposes the patients towards breakdown of B cell tolerance.

Why such B cell autoimmune phenomenon takes place in the absence of ***CFHR1*** , what immune circuits are broken to lead to such malfunction remains to be explored. But it is interesting to note here that analysis of the frequency of *CFHR1* deficiency amongst healthy individuals have revealed an occurrence of 5-10%. This means that the patients either have some further mutations that in combination with the absence of *CFHR1* predisposes them to autoimmunity or that the healthy individuals harbor some protective mutation/mechanism such that the ill-effects of not having *CFHR1* is tolerated by the body. aHUS patients suffering from acute kidney disease as a result of *CFHR1* deficiency have severe underlying inflammation which in many cases may occlude identification of players that are responsible for initiating immunological malfunctions. In the asymptomatic individuals the absence of such inflammation might ease identification of immunological pathways crucial for breakdown of B cell tolerance when there is no *CFHR1*. Thus, we decided to first examine the immune status of healthy individuals lacking *CFHR1* gene. For this we collected buffy coats (mononuclear cell enriched fraction of blood). Peripheral blood mononuclear cells (PBMC) were isolated from buffy coats by layering them on ficoll and stored at -80C for future use. Serum separated from these samples were also stored under similar conditions. Initial analysis identified 4 individuals who lacked the *CFHR1* gene, in agreement to the 10% frequency of occurrence of *CFHR1* homozygous deletion in healthy population. An ELISA for anti-CFH autoantibody revealed that sera collected from CFHR-/- healthy individual lack significant anti-CFH antibody titer (Figure 2).

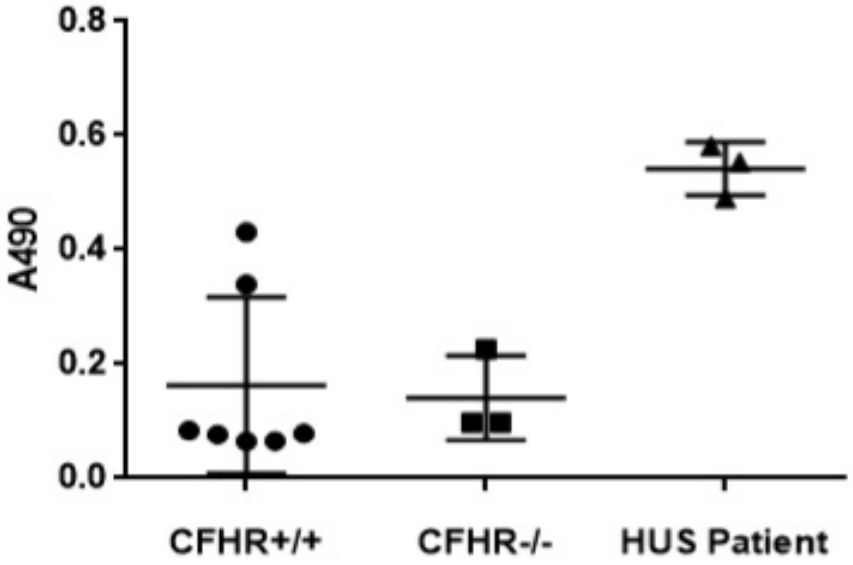

Figure 2. Anti-CFH autoantibody titers in CFHR-1- sufficient, CFHR-1-deficient healthy individuals and aHUS patients.

We have analyzed different immune cell subsets in these individuals, like plasmacytoid and myeloid dendritic cells, classical, patrolling and inflammatory monocytes, CD4 and CD8 T cells (both naïve and memory), B1B and conventional B cells. We have also calculated both, the proportion of these subsets as well as absolute cell numbers. Since CFHR1 deficiency in patients leads to autoantibody production, thereby revealing a clear breakdown of B cell tolerance mechanisms we also focused on assessing various developmental stages of B cells like immature and transitional B cells, stages crucial for deletion of self-reactive B cell clones. The initial data showed some differences between CFHR1+/+ and CFHR1-/- individuals (Figure 3) but since the sample size is small, definite

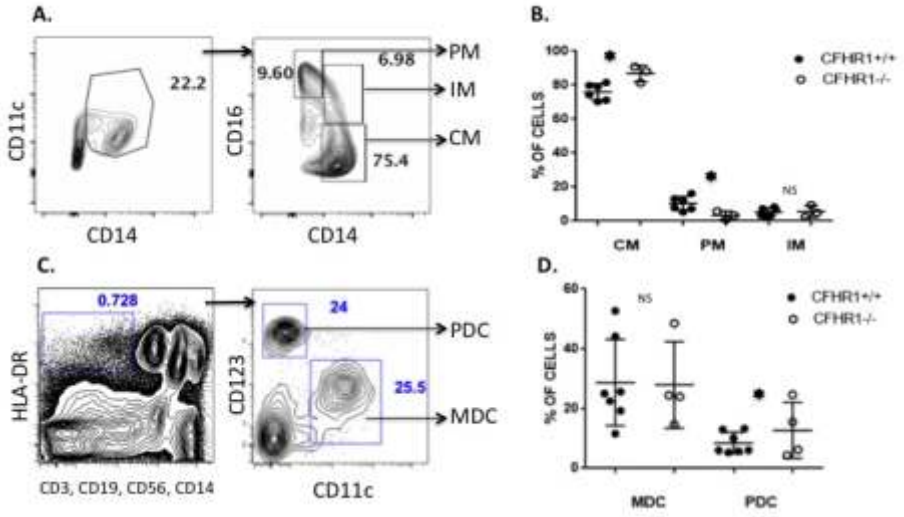

Figure 3. Immunophenotyping of monocytes and dendritic cells (DC). A. Gating strategy for monocyte subsets (PM= patrolling monocytes; IM= inflammatory monocytes; CM= classical monocytes). B. Graphical representation of monocyte subsets from control and CFHR1-deficient individuals. C. Gating strategy for DC subsets (PDC= plasmacytoid DC; MDC= myeloid DC). D. Graphical representation of DC subsets from control and CFHR1-deficient individuals. B and D are data from 6-7 control and 4 CFHR1-/- individuals.

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conclusions can only be drawn after accumulation of more data sets to attain statistical relevance.

Besides proportion of immune cells, it is often the capability of these cells to respond successfully to antigenic challenge that is more widely affected by mutations. Hence, we are standardizing assays to measure functionality of various subsets of immune cells. All assays were performed using frozen PBMC that were given rest in complete media. For T cells, we try to analyze their ability to undergo activation and proliferation following mitogenic stimulation with phytohemagglutinin or using plate- bound anti-CD3 and anti-CD28 to mimic T cell receptor engagement by antigens (Figure 4). Death following such activation was also measured. Memory T cells have lower threshold for activation and can be stimulated to produce cytokines following a 4hr exposure to PMA and Ionomycin. We assayed an array of cytokines like, IL13, IL17, GMCSF, IFNγ and TNFα produced by CD45RO negative memory T cells. Monocytes and dendritic cells function as professional antigen presenting cells and are extremely efficient at phagocytosing antigens and then presenting them to T cells

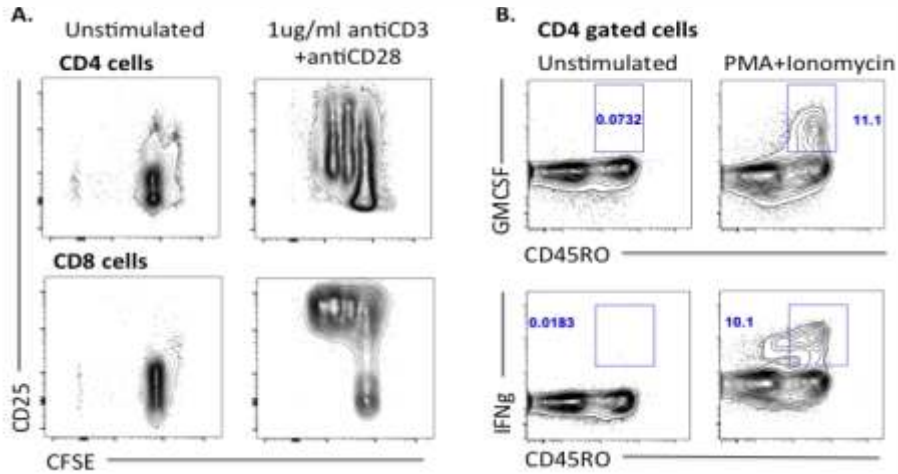

Figure 4. T cell proliferation and cytokine secretion assay. A, CFSE dilution and CD25 upregulation on CD4 and CD8 cells following stimulation. B. GMCSF and IFNγ production by CD4 memory (CD45RO+ve) cells on activation.

To assess the phagocytic ability of these cells, plate adhered CD14 rich cellular fraction was given fluorescently tagged bacteria, and after 2hr the amount of bacteria taken up by the cells was measured using flow-cytometry. Apart from T cells and monocytes we are also standardizing the functionality of B cells by assessing their ability to respond to TLR9 activation by proliferating and differentiating into antibody secreting plasma cells.

CFHR proteins have structural similarity to CFH and bind to similar structures on host cells but lack the ability to inactivate C3. Hence they function as competitive inhibitors of CFH. The absence or presence of CFHR can therefore control the amount of CFH deposited on host cell surfaces. CFH deposition has many consequences apart from its most appreciated role in controlling complement activation on host cells. CFH prevents uptake of apoptotic cells, enhances chemotaxis and phagocytic ability of monocytes and dendritic cells. We observed that CFH is bound to monocytes in higher proportion than other immune cells and also

among monocytes their levels are highest on classical monocytes (Figure 5). It would be interesting to assess if CFH deposition on monocytes vary in CFHR+/+ and CFHR-/- individuals and the consequences of excess CFH deposition, if any, on classical monocyte function.

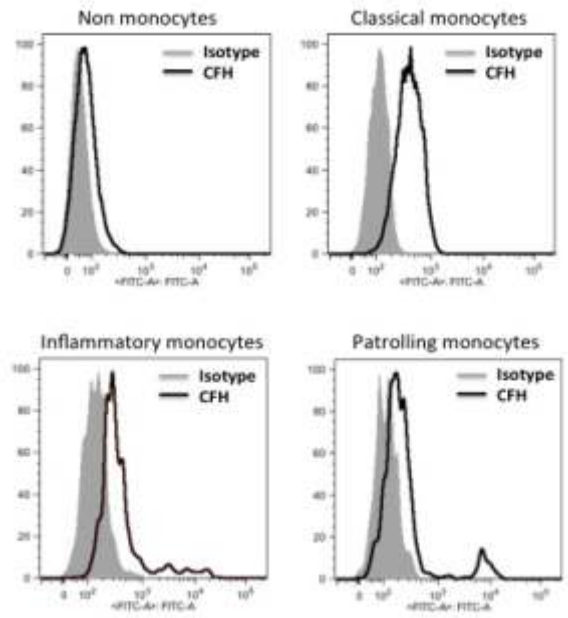

Figure 5. CFH levels on different monocyte subsets.

Future plans

We plan to proceed with this project under two phases: first, examination of adult CFHR1-deficient but anti-CFH antibody negative blood bank cells with assays for relevant evidence of immune dysfunction and then to use those results in the second phase to make predictions and hypotheses and then test the same in anti-CFH autoantibody positive pediatric samples with or without kidney disease. The initial hypothesis is that since CFH prevents proper uptake of apoptotic cells, hence imbalance of CFHR-CFH levels in aHUS patients with CFHR1 deficiency might contribute to improper clearance of apoptotic debris, thereby chronically activating phagocytic cells (responsible for removing the debris) and releasing proinflammatory cytokines in excess. In the absence of CFHR1, an excess load of apoptotic debris coated with high levels of CFH would result in activation of anti-CFH B cell clones in the periphery. This would explain the presence of high titers of anti-CFH autoantibody in such patients.

Publications

Original peer-reviewed articles

1. Heesters BA, Das A, Chatterjee P and Carroll MC (2014) Do follicular dendritic cells regulate lupus-specific B cells? ***Mol Immunol*** 62: 283.

Scientific Reports

Structural Biology of Host-Microbial Interactions in Health and Diseases

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Susheela Kushwaha (upto Aug 2014)

Research Fellows

Priyanka Chaurasia

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Abhin Kumar Megta

Rajnesh Kumari Yadav

Research Theme

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

Objectives

- Investigate pilus architecture, assembly and adhesion process in beneficial and pathogenic bacteria.
- Understand bacterial pili-mediated adhesion strategies in initiating host interaction

Progress of work during the current reporting year 2013-2014

Pili or fimbriae are hair-like surface organelles assembled by both Gram-negative and positive bacteria. In pathogenic bacteria, pili have often been implicated in mediating adhesion and initiating host interactions for pathogenesis. Substantial structural knowledge exists for the pili from Gram-negative pathogens, while it has begun to emerge since 2007 for pili from Gram-positive pathogens. Presence of pili has also been recently observed in beneficial bacteria (e.g. probiotics) and shown to mediate host interactions for probiosis. Relatively little is understood about pili from beneficial bacteria, and a structural knowledge has yet to be achieved for detailed understanding of their structure, assembly process, mode of adhesion, and host interactions.

We have chosen *Lactobacillus rhamnosus* GG as a model system to study pilus structure and function in beneficial bacteria as it is one of the most extensively studied probiotic strains for its health promoting-effects and often used as reference strain in the clinical trials.

Genome of *L. rhamnosus* GG contains two pilus gene clusters (*SpaCBA* and *SpaFED*) for pilus biogenesis similar to several Gram-positive pathogenic bacteria. The SpaCBA pilus cluster contains genes of a shaft pilin SpaA, two ancillary pilins SpaB and SpaC, and a pilin-specific sortase SrtC1. Similarly, the *SpaCBA* pilus cluster consists of genes of a shaft pilin SpaD, two ancillary pilins SpaE and SpaF, and a pilin-specific sortase SrtC2. The function of pilin subunits and their location in the assembled pilus fiber of the *L. rhamnosus* GG has

Scientific Reports

been proposed based on immunogold electron microscopic studies and in comparison with pili from Gram-positive pathogens. The repeating SpaA forms the pilus shaft. The SpaB and SpaC usually found at the base and tip of the pilus fiber might involve in cell wall-anchoring and adhesion, respectively. Interestingly, in contrast to the pili from several Gram-positive pathogens, the SpaC (and, to a lesser extent, SpaB) is distributed sporadically throughout the shaft of the SpaCBA pilus. It is thought that this distribution could enhance adherence to the intestinal mucosa and epithelial layer, and thus increase the relative longevity and transient colonization of *L. rhamnosus* GG cells in the gastrointestinal tract (GIT). The SpaFED pili are yet to be observed in the laboratory conditions despite the presence of the *SpaCBA* gene in the genome. The SpaFED pili may be produced in intestinal tract, similar to the Tad pilus in *Bifidobacterium brevei* or in standard conditions by over expression of pilus operon as demonstrated in *L. Lactis* IL1403.

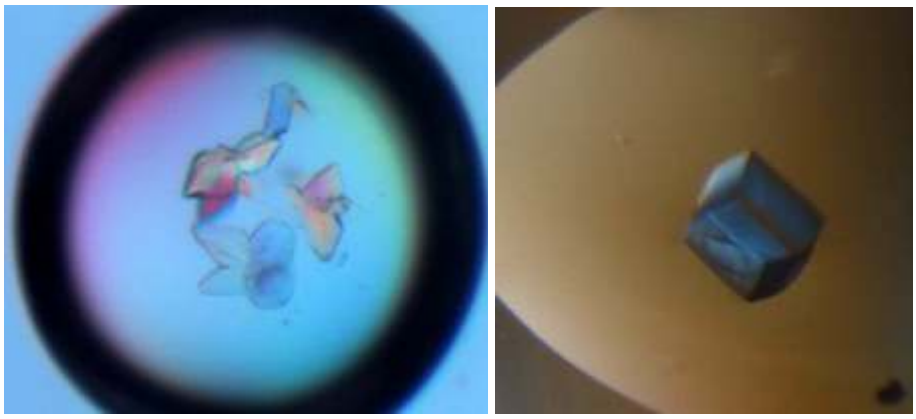
We continued our studies on pilins and sortases of *L. rhamnosus* GG SpaCBA and SpaFED pilus, towards understanding the mechanisms of pilus assembly and adhesion. Recombinant clones for these proteins were obtained from Dr. Airi Palva's group, University of Helsinki, Finland.

Preliminary crystallographic analysis of SpaA, a 30 kDa shaft pilin subunit in SpaCBA pili, was reported earlier. The native crystals obtained from the optimized condition (0.35 M trisodium citrate and 14% PEG 3350) were diffracted to 2.0 Å at synchrotron source BM14, ESRF (Grenoble, France). The SpaA crystals belonged to space group *C*2, with unit-cell parameters a = 227.9 Å, b = 63.2 Å, c = 104.3 Å, β = 95.1°. Matthews coefficient (V_M = 2.44 $Å^3$ Da^{-1}) calculation with 50% solvent content suggested that there could be five molecules in the asymmetric unit. Initial attempt to locate five SpaA molecules by molecular replacement was unsuccessful mainly due to its limited sequence identity with known structures. Attempts to solve the structure by selenium or sulfur single-wavelength anomalous dispersion (SAD) method was not feasible because there are no methionine or cysteine residues in the recombinant SpaA. However attempts were made to introduce few methionine residues by mutation for Se-SAD phasing, but the quantity of purified mutant protein was not enough for crystallization, likely due to solubility issues. Numerous attempts to use conventional heavy-atoms and also halides soaking were often resulted in crystal cracking, weak signal, and poor diffractions. Our final attempt to solve the structure by combination of limited proteolysis and domain-based molecular replacement were successful, and it produced traceable electron density map. Model building and refinement are being carried out.

SpaD, a 50 kDa shaft pilin subunit in SpaFED pili, has been purified, and crystallized by vapour diffusion methods in several conditions. However the SpaD crystals showed anisotropy with a streaky diffraction pattern during the in-house data collection. The limited proteolysis approach was used, while attempts are being made for obtaining good diffraction pattern by using various additives, cryo-protectants, dehydration techniques, etc. After limited proteolysis treatment, crystallization attempts were made, and crystal growth was observed in two conditions. One of the conditions was further optimized to obtain diffraction quality crystals. The crystals from the condition containing 0.18 M Lithium Sulfate and 22% PEG 3350 were diffracted to 2.2 Å at home source and belonged to orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 50.47 Å, b = 82.81 Å, c =

149.06 Å. Molecular weight estimation by gel-filtration, SDS-PAGE and mass spectrometric methods suggested that the crystallized fragment of SpaD is about 35 kDa. The Matthews coefficient (V_M = 2.44 $Å^3$ D^{a-1}) calculation with 50% solvent content suggested that there are two molecules in the asymmetric unit. Attempts to solve the phase problem using halide quick soaking method at home source by SAD technique has yielded interpretable electron density map. Currently, the model building and refinement are being performed.

SpaC, a 90 kDa tip pilin subunit in SpaCBA pili, has been purified by affinity and gel-filtration chromatographic techniques. Initial conditions for SpaC crystal growth have been identified after thorough screening, and optimization are being pursued for obtaining single large-size crystals.



Crystals of SpaA and SpaD after limited proteolysis.

Future Plans

Structural and functional investigations of pilin subunits and sortases from ***L. rhamnosus*** GG will be continued. Structural studies on pili from primary colonizers of oral biofilm (plaque) will be initiated. Pili from these primary colonizers mediate adhesion to host tissues and interaction with secondary colonizers for the oral biofilm formation. This will help us in making comparisons and understanding common and specific strategies of beneficial and pathogenic bacteria.

Publications

Original peer-reviewed articles

1. Chen C, Krishnan V, Macon K, Manne K, Narayana SV and Schneewind O (2013) Secreted Proteases Control Autolysin-mediated Biofilm Growth of Staphylococcus aureus. ***J Biol Chem*** 288: 29440.

Scientific Reports

Studies on Biology of Infectious and Idiopathic Inflammation of the Gut

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Research theme

Wellness of an individual starts from the health of the gut. Using a model intracellular gastric pathogen, Salmonella Typhimurium, we aim to understand the molecular mechanisms that shape infection, inflammation and auto-immune disorders of the gut.

Objectives

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

Progress of work during the current reporting year 2013-14

The mucosal surfaces of the intestine are in constant threat of innumerable chemical and biological agents. The status quo of the host genetic make up, the commensal microflora composition and the environment together account for the overall health of the intestine and enable them to confront infectious agents. Among the various microbial threats that pose a challenge to the host health, a frequent casual agent of food borne illnesses is Salmonella Typhimurium (ST). The disease is called gastroenteritis that results in massive neutrophil infiltration at the site of infection. Remarkably this is a phenotype also seen in several forms of autoimmune disorders such as Crohn's disease (CD) and ulcerative colitis (UC). Several molecular markers of acute inflammatory state (such as presence of neutrophil-chemotractant hepoxilin-A3, upregulation of multidrug resistant proteins) are also shared between these diseases. In our lab we have been investigating pathways of the gastrointestinal inflammatory conditions using model pathogen ST to unearth novel mechanisms that are key in the states of inflammation and disease. Specifically, we had earlier (year 2012-2013) identified that host SUMOylation, a post-translational modification pathway, was significantly altered by ST during infection. The SUMO machinery, similar to

the ubiquitylation machinery, utilize three enzymes that includes an activating enzyme E1, a conjugating enzyme E2 (also called as Ubc9) and several ligating enzymes called E3. In a stepwise manner these enzymes conjugate one of three the SUMO substrates (SUMO1 SUMO2 or SUMO3) to lysine residue target proteins that have a SUMO motif. The SUMO modification could essentially alter several features of the target protein including its localization, function and its ability to interact with other proteins.

Investigations done by us last year (2012-2013) essentially revealed that ST significantly altered the host SUMOylation in a dynamic manner over the course of infection. The SUMO alteration required live ST with functional Type Three Secretion System (TTSS). Moreover, one of the host targets utilized by ST to modulate the host SUMO machinery was the E2 enzyme Ubc9. We observed that during ST infection Ubc9 protein levels of the host cells were depleted.

In the current year (2013-2014) we first tried to examine if the observed ST mediated SUMO

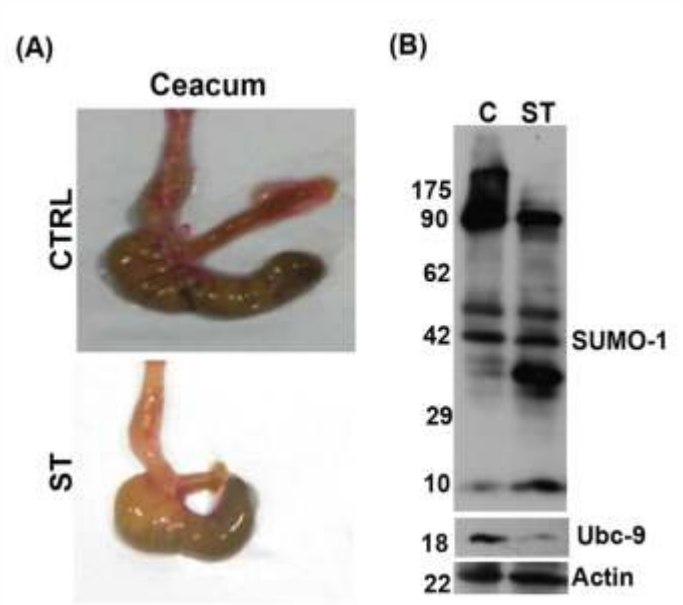


Figure 1. ST down regulates host SUMOylation levels in vivo. (A) Gross morphology of caecum of mice with (bottom) and without (top) infection with SL1344. (B) Immunoblot analysis of lysates prepared from mouse epithelial cells isolated form the proximal colon. Beta actin was used for normalization.

Immunoblotting of the epithelial cell preparation from colon (proximal) revealed that there was a decrease in the global SUMO1 profile in the infected samples in comparison to the uninfected controls. Moreover the Ubc-9 levels were also affected in the infected samples (Figure 1) indicating that the mechanism of SUMO alteration that was seen in cell culture model is also true for the murine model. In totality, these data point out to a novel mechanism utilized by ST that may have a wide impact on the host cell machinery. Next we probed into the mechanism of ST mediated host SUMOylation alteration. Ubc9 being the only enzyme catalysing the crucial step of SUMO conjugation we probed deeper into mechanism of ST mediated Ubc9 depletion.

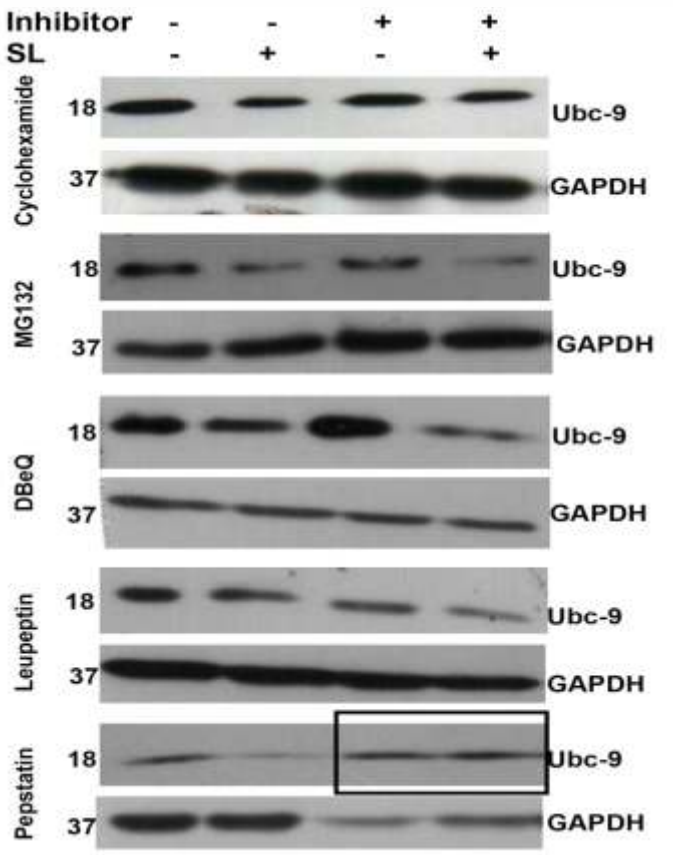


Figure 2. Ubc9 protein degradation depends on Aspartyl proteases. Immunoblot analysis of HCT8 cells with or without infection with ST at different time points, probing for Ubc9. Mechanism of Ubc9 downregulation was assessed via inhibitors of protein synthesis and various cellular degradation pathways including Proteasomal (MG132, Autophagic (DBeQ, Lysosomal (Leupeptin, and Cytosolic (Pepstatin, were used (as indicated in the panels).

To examine if SUMO alteration was due to active degradation or inhibition of protein synthesis machinery, we carried out infection in the presence of the eukaryotic protein synthesis inhibitor Cyclohexamide (CHX). CHX did not prevent the Salmonella-mediated decrease in Ubc9 levels (Figure 1) indicating that ST does not affect translation of Ubc9 per se but rather induce its degradation. Protein degradation within the cells is primarily carried out in the cytosol via the Ubiquitin mediated proteasome machinery and also via some proteases; as well as in the lysosome via the hydrolytic enzymes. We individually tested the role of each of these pathways using specific pharmacological inhibitors. Infection of HCT-8 cells with ST in the presence of MG132, an inhibitor of the proteasome degradation machinery failed to rescue Ubc9 depletion (Figure 2).

Leupeptin which is an inhibitor of Lysosomal proteases and DebQ, an autophagy inhibitor, also had no effect on the Ubc9 depletion (Figure 2). However, Pepstatin A methylester, an aspartyl protease inhibitor, was able to completely reverse the effect of ST mediated Ubc9 depletion (Figure 2). These data indicated that the ST mediated Ubc9 depletion was independent of the 26S proteasome machinery, lysosomal degradation, including autophagy dependent degradation mechanisms. Rather the mechanism was a cytosolic

pathway that depended on aspartyl-proteases. The possibility of other cellular mechanisms also involved in ST mediated Ubc9 depletion can not be ruled out and would therefore need more investigations.

Inflammation is an intrinsic aspect of Salmonella infection and favours the growth of the bacterium in the intestine along with aiding it in competing with intestinal microbiota. Moreover, several regulators of inflammatory cascade have been previously demonstrated to be dependent on SUMOylation for their proper function. The key players among these involve p65/RelA, a component of the master regulator Nuclear Factor kappa B (NFkB) and its repressor, IkBα. Thus, we embarked on studies to understand the impact of ST mediated SUMO alteration on the inflammation pathway of the host. Comparative expression profiling of NFkB-related genes was carried out in ST infected host cells that were either upregulated for their SUMO machinery (by over-expression of Ubc9 using plasmid pCDNA3/Ubc9) or were left unperturbed (controls). Our aim was to oppose the ST-mediated SUMO machinery down-regulation and then examine its consequence in the outcome of inflammation. In our approach we mainly compared the relative expression of genes in ST infected cells versus cells that have been upregulated for their SUMO machineries (Ubc9).

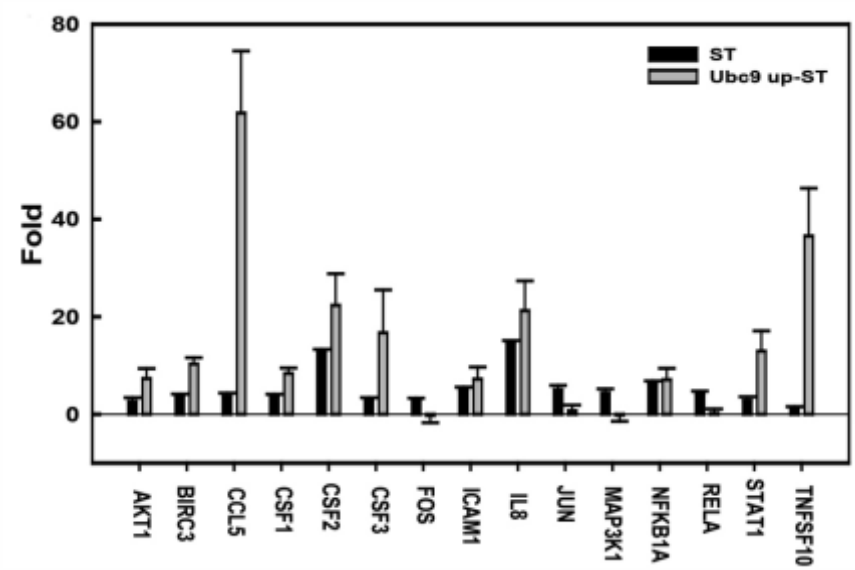


Figure 3. Contribution of SUMOylation towards inflammation was assessed using a PCR Array for NFkB related genes by comparing fold change (in comparison to control uninfected HCT8 cells) in mRNA levels of ST infected HCT8 cells with and without upregulation of SUMOylation machinery by overexpression of Ubc-9.

We were able to get upto 6-8 fold upregulation of Ubc9 expression compared to the control cells (Figure 3). cDNAs of untreated (control), control-infected (C-ST) and Ubc9 upregulated infected samples (Ubc-9-up ST) were used in 96 well plate of human NFkB signalling pathway PCR Arrays (Qiagen RT2 NFkB PCR arrays) and relative gene expression was studied. The relative expressions of several of these genes was drastically affected in Ubc-9-up ST cells (Figure 3 grey versus black bars). Volcano plots displayed several genes to be upregulated during ST infection, notable among them being IL8, CSF2,

Scientific Reports

TNF, ICAM, JUN and NFKB1A (Figure 3A). Many of these have been previously reported to be activated during ST infection.

Fold difference of gene expressions in Ubc9 overexpressed, ST infected (Ubc9-up-ST) cells when compared with control group, were significantly altered in contrast to infected but non-perturbed (C-ST) cells. This included a change in IL-8 levels from 3.5 fold in C-ST to 4.2 fold in Ubc9-up-ST, CSF2 from 3.2 fold to 4.3 fold. Moreover, in Ubc-9-up-ST (Figure 3) several additional genes were also were seen to be upregulated. Key candidates among these were (a) signalling genes such as STAT1, TLR3, AKT and BIRC3, (b) cytokine encoding genes such as IL8, IL1alpha, TNF (c) NFkB regulated genes such as CCL5, CSF3 and (d) components of NFkB such as RelA, displayed upregulation. It was notable that the observed fold changes were also statistically significant. We looked at the protein levels of some of these genes such as RelA and STAT1 and found our results to concur. Thus, these data indicated that SUMOylation dependent control of inflammatory genes is an integral part of ST infection.

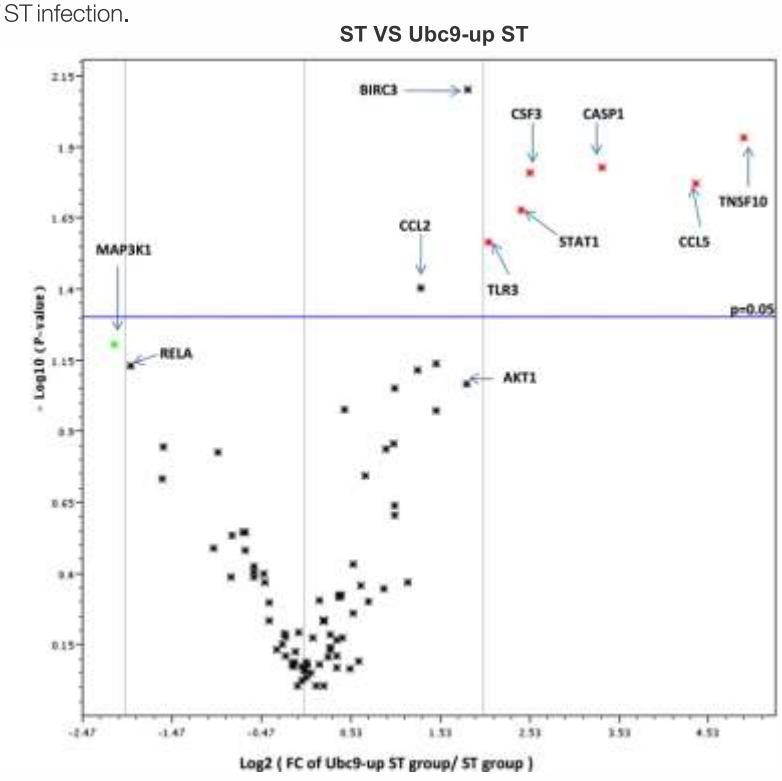
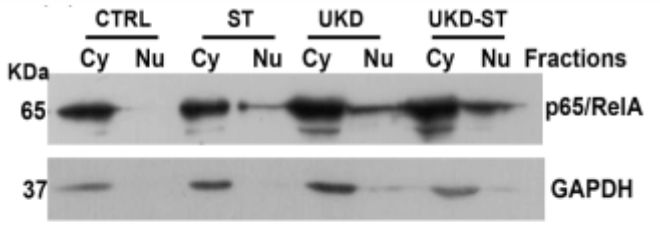


Figure 4. Comparative expression analysis of inflammatory pathway genes. Volcano plots of fold change upon infection with ST and Ubc9 overexpressing cells infected with ST. Genes on the top right are the ones that are upregulated upon the Ubc9 overexpression and ST infection. These changes are statistically significant ($p < 0.05$).

To gain a deeper understanding of the molecular mechanism behind the observed control of the process of inflammation by SUMO machinery we examined the localization and protein levels of RelA in detail. Importantly even slight changes in the function of these master regulators have been demonstrated to be extremely deleterious to the cell leading to inflammatory disorders and cancer. In case of ST infection NFkB are known to be up-regulated and are important mediators of the observed inflammation. We conceptualized that if ST has the ability to modulate the inflammatory pathway of the host using SUMO

machinery then components of the NFkB factor, such as RelA, would be the key targets. Several of the members of the NFkB pathway have been shown to be modulated by SUMOylation. However, the regulation of these factors by SUMOylation is specific only to certain types of trigger. For instance, NFkB component p65/RelA has recently been demonstrated to be SUMOylated upon TNFα treatment but not leptomycin B. Given these, we investigated the requirement of SUMOylation in regulation of NFkB in the context of ST infection.

Since the activity of p65 requires it to translocate into the nucleus, we initially looked at both cytoplasmic and nuclear localization of p65 upon perturbation of SUMO machinery by sub-cellular fractionation of nuclear and cytoplasmic pools of RelA. Almost the entire pool of RelA was localized outside the nucleus in untreated cells (Figure 5). Upon infection, as also observed by others, a fraction of p65/RelA re-localized into the nucleus. In case of Ubc9 KD cells however, even without infection there was RelA localization inside the nucleus (Figure 5). In the Ubc9 KD cells, ST infection led to further increase in the nuclear pool of RelA, which was almost double the amount of what is seen in a regular infected cell (Figure 5).



	Control		ST		UKD		UKD-ST	
Intracellular Fraction	Cyto	Nuc	Cyto	Nuc	Cyto	Nuc	Cyto	Nuc
Densitometric Intensities	95.98	20.70	83.78	26.83	110.7	34.58	105.37	38.48
Cyto/Nuc Ratio	4.63		3.1		3.2		2.7	
Nuc/Total Ratio	0.177		0.24		0.23		0.26	

Figure 5. Nuclear and Cytoplasmic fractions of control and Ubc9 KD cells with and without ST infection were immunoblotted for p65 (top) or GAPDH (representing the cytoplasmic fraction, bottom panel). Densitometric analysis was carried out using Image Quant tool and reported in the table below.

This meant that, RelA re-localization which is one of the events needed for its function may be under the control of SUMOylation (Figure 5 Table - nuclear/total fraction). Comparison of cytoplasmic/nuclear ratios of RelA suggested that perturbation of SUMOylation results in disruption of the fine balance of partitioning of RelA.

Future Plans

- Analysis of novel protein factors (identified by expression analysis and Mass-spectrometric analysis) and pathways involved in ST mediated inflammation
- Investigations of molecular mechanisms of ST mediated SUMO alterations
- Examining the possible role of SUMO pathway in IBD and colon cancer in patient samples and in animal model system

Structural Biology of Regulatory Events in Physiological Processes

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Research Theme

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

Objectives

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

Progress of work during the current reporting year 2013-14

The hallmark of acquired immune system is remarkable specificity in its recognition repertoire that not only counters the invading pathogens but also ensures self and non-self discrimination. Since early 1930's it is known that the immune system is highly specific and can distinguish the subtle differences in the antigens. However, degeneracy in immune recognition is often observed. While, the degeneracy to similar or related antigen is expected, it seems to extend to chemically distinct antigens as well. Cross-reactivity as seen in both germline and affinity-matured antibody is in defiance to the 'rule of specificity'. What governs the existence of such a dichotomy in specificity and degeneracy in the humoral response? We have extensively analyzed in the past the degeneracy of antigen recognition by polyclonal as well as monoclonal antibodies, also called molecular mimicry, using a peptide (DVFYPYPYASGS) and a sugar (methyl α- D-mannopyranoside). An anti-sugar monoclonal antibody (2D10) was shown to be recognizing both sugar and its mimicking peptide with equivalent affinity (*Goel et al., (2014) J Immunol 173:7358; Krishnan et al., (2008) Biophys J 94:1367*). The structures of 2D10 in apo and the antigen-bound forms (with the sugar as well as the peptide) showed that no conformational flexibility in CDRs of 2D10 existed, instead it was evident that the plasticity in the interaction had helped in the manifestation of molecular mimicry (*Tapryal et al., (2013) J Immunol, 191:456*)

Therefore, in order to decipher the fine structural elements associated with this degenerate specificity potential, we explored other sugars and peptides which can bind to the 2D10

Scientific Reports

antibody with comparable affinities. Crystallographic analyses of 2D10 bound to five different sugars (methyl α D glucopyranoside, α D lactose, α1-3-Mannobiose, α1-6-Mannobiose, α1-3, α1-6-Mannotriose) were carried out at 2.5 Å, 2.5 Å , 2.1Å, 2.75 Å and 1.7 Å, respectively. The comparisons among these structures have provided interesting insights underlying the basis of specificity in molecular recognition. The antigen combining site for sugars is constituted of CDRs H3, L1 and L3 only. All the five sugars have an overlapping primary binding site (equivalent to the methyl α-D-mannopyranoside interacting region). Primary binding site has 8 interacting residues (K100, N101, Y102, S105, Y176, S235, Y236 and Y240) and all the non-reducing sugars which are accommodated in the primary site utilize the interactions from these 8 residues only. This primary sugar binding site has been shown to accommodate same/similar as well as dissimilar sugars by utilizing plasticity in the interacting residues available in the antigen combining site. The second sugars of the similar disaccharides (α-1-3-Mannobiose, α-1-6-Mannobiose) have been adjusted in the same direction but with utilizing different sets of interacting residues of the antibody paratope. However, the second sugar of dissimilar disaccharide (lactose in comparison to α-1-3-Mannobiose, α-1-6-Mannobiose) exploits different paratope space altogether. The trisaccharide (α-1-3, α1-6-Mannotriose) was accommodated in the same site by differential positioning of the second and third sugar rings in the antibody paratope (in comparison to all disaccharides) as well as by utilizing conformational flexibility in the paratope region (mainly in CDR L1). This study had demonstrated that an affinity matured antibody can utilize atleast three different strategies in order to accommodate structurally similar/dissimilar sugars.

We have addressed the problem of antibody degeneracy by bioinformatics approach as well. Over the years crystallographic studies on antigen-antibody interaction have strengthened the observation that there is no perfect apposition in the established paradigm and the emerging data. Nearly 3500 antibody structures have been determined till date of which about 658 structures delineate the full extent of antibody-antigen interactions. It is anticipated that a coherent analysis on global data would aid in understanding the pliable nature of an antibody. Such analyses will help understand if there is a pattern that fits the current models for speculating antigen recognition. This in turn will help understand the evolution and versatility of an antibody. We have restricted our data to the crystal structures which have been refined at or better than 3Å resolution from RCSB PDB. An exhaustive search of the database yielded a total of 658 structures. These were then subjected to a number of filters to exclude humanized or chimeric structures and eliminate redundancy. Data mining was segregated for mouse and human antibodies. Our objective was to see if there is any apparent behavior in antibodies originating from common ancestors while exercising their recognition potential for the limitless antigenic space. For this, the coding genes of the antibodies were traced. Since an antibody's genetic machinery is composed of V, D and J gene segments that recombine to form the functional molecule, germline genes of the structures were identified. The final dataset was composed of 38 germline origin encompassing 148 structures from mouse and 14 germline origin encompassing 76 structures from human. Analyses of these structures have shown that both germline and mature antibodies exhibit degenerate specificity while interacting with the milieu of antigens. Besides, majority of the mature antibodies originating from a common ancestor were seen to recognize chemically and structurally distinct antigens. As a matter of fact, the dataset also contained some antibodies that showed polyspecificity. Further analyses are in progress.

As a part of our structural proteomics of seed allergen studies, we had reported earlier the crystal structure of SM80.1 from eggplant (***Solanum melongena***) and preliminary studies

on other protein, SM80.2, from the same plant. Structure of SM80.2 has now been determined by ab initio phasing utilizing eight inherited sulphur atoms (cysteine). Experimental anomalous intensities were used by the PHENIX package to obtain initial phase information. Program SOLVE successfully identified in total seventeen sulphur sites from the Bijvoet pair differences. A preliminary model was built automatically by Autosol. At the current stage of refinement, the overall R factor of 25.80 and free R factor of 28.83 are achieved. Ramachandran plot has 174 residues (98.31%) in allowed region and 2 (1.13%) in preferred. Only one residue is present in outlier. The overall structure of SM80.2 consists of 177 residues with continuous density. It consists of two monomer in the asymmetric unit. This high resolution data helped in extracting the complete amino acid sequence of SM80.2. Sequence analysis of SM80.2 showed 80 % sequence similarity with non-specific lipid-transfer protein AP10-like from ***Solanum lycopersicum***. A peculiar unexplained continuous density was also found during refinement. As this protein is a non specific lipid transfer protein and thus is involved in transferring of lipids, we expect this continuous unexplained density may correspond to lipid moiety bound to protein. Also this lipid binding could be linked to allergenic activity shown by non specific lipid transfer protein.

Future plans

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

Publications

Original peer-reviewed articles

1. Khan T and Salunke DM (2014) Adjustable locks and flexible keys: plasticity of epitope-paratope interactions in germline antibodies. ***J Immunol*** 192: 5398.
2. Gill J, Jayswal P and Salunke DM (2014) Antigen exposure leads to rigidification of germline antibody combining site. ***J Bioinform Comput Biol*** 12: 1450006.
3. Sharma R, Lomash S and Salunke DM (2013) Putative bioactive motif of tritrpticin revealed by an antibody with biological receptor like properties. ***PloS One*** 8:e75582. [in press last year, since published].

Reviews/Proceedings

4. Salunke DM, Gill J and Dwevedi A (2014). Comparative structural proteomics of allergenic proteins from plant pollen. ***J Ind Inst Sci*** 94:119.

Scientific Reports

Pathophysiology of Thrombosis in Disease Conditions

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Research Theme

Our objective is to delineate the complex pathophysiology of thrombosis (intravascular clot formation) and blood vessel blockage in different diseases. Current research projects aim to elucidate the following mechanisms: 1) how intravascular hemolysis increases the risk of thrombosis in hemolytic diseases such as paroxysmal nocturnal hemoglobinuria (PNH) and hemolytic uremic syndrome (HUS); 2) hypoxia mediated regulation of erythropoiesis, hypercoagulation and thrombosis at high-altitude; and 3) pathophysiology of thrombocytopenia in dengue infections.

Progress of work during the current reporting year 2013-14

1) We have demonstrated that the extracellular hemoglobin (Hb) significantly increases the binding of von Willebrand factor (VWF) to platelets and promotes aggregation and thrombus formation under shear flow conditions. Hb binds to the active-form of VWF at A1 and A2 domains and to the N-terminus of glycoprotein 1bα (Gp1bα) subunit of VWF receptor on platelets. We show that Hb potentiates VWF-GP1bα binding by bridging these two proteins. A synthetic peptide derived from GP1bα N-terminal domain inhibited Hb-platelet interactions and the prothrombotic effects of Hb. We postulate

Scientific Reports

that this is the principal molecular basis of increased risk of thrombosis in hemolytic patients. The analysis of plasma of patients with paroxysmal nocturnal hemoglobinuria (PNH), a prototype of intravascular hemolytic anemia with recurrent thrombotic complications, supports this concept. We found that PNH patients' plasma contains significant amount of Hb that is bound to microparticles (MPs) as well as to VWF and GP1bα, a potent prothrombotic stimulus promoting platelet aggregation. We have communicated this work for publication.

2) We have quantified the gene expression as well as plasma protein level of different hypoxia responsive prothrombotic factors in Leh Tibetans (LT) who live at an altitude of 5000m from sea level under extreme hypoxic conditions (partial oxygen pressure about 50% of the sea level). In LT, we observed increased level of the early growth response factor [Egr-1, which promotes tissue-factor (TF) synthesis], and TF protein when compared with Delhi Tibetans (DT). Also, we observed increased expression of plasminogen activator inhibitor (Pai-1) and thrombin activatable fibrinolysis inhibitor (TAFI) in LT. Simultaneously by upregulating these fibrinolysis inhibitors (PAI-1 and TAFI) we observed less fibrin degradation in LT than DT. These observations in LT further lead us to enquire whether the upregulation of these pro-thrombotic/coagulative factors is a normal or a clinical phenomenon in response to extreme hypoxic conditions. We have communicated this work to Leh Symposium 2014.

   In another work, a multi-institutional collaborative project, we have described a high-frequency missense mutation in the ***EGLN1*** gene, which encodes prolyl hydroxylase 2 (PHD2). In general, wild-type PHD2 increases the degradation of HIF under normoxic conditions. However, we found that this mutation in ***EGLN1***, c.[12C>G; 380G>C],promotes increased degradation of hypoxia-inducible factors (HIF) under hypoxic conditions. Further, we show that hypoxia stimulates the proliferation of erythroid progenitors (from persons who do not possess this mutation). Whereas the proliferation of erythroid progenitors (from the person with c.[12C>G; 380G>C] mutation in ***EGLN1***)is significantly impaired under hypoxic culture conditions. Thus the c.[12C>G; 380G>C] mutation abrogates hypoxia-induced and HIF-mediated augmentation of erythropoiesis. This observation provides an insight into the molecular mechanism of the observed protection of Tibetans from polycythemia at high altitude. This work is published in the ***Nature Genetics***.

3) We have also made progress in elucidating the mechanism(s) underlying the pathogenesis of thrombocytopenia in dengue infections. We are focusing mainly on two major pathways that decrease platelet counts in circulation during infections. We are standardizing the gene expression profiles of the transcription factors (such as Runx1, Gata1, Fli1, c-Myb, Fog-1 and Pu-1) and stimulatory factors (such as TPO) that regulate the differentiation of hematopoietic stem cell (HSC) to megakaryocyte (MK) and further to platelets. We are planning to measure the above gene profiles in patients with dengue infection (DNV serotype 1-4), who develops severe thrombocytopenia compared to mild/no-thrombocytopenia. We are also focused on determining the ligands on MK/platelet surface that bind to antibodies for clearance from circulation. Also we will be investigating the plasma pro-thrombotic/coagulative factors that promote MK/platelets aggregation/ agglutination and depletion from circulation of dengue patients with thrombocytopenia.

Future plans

Hypoxia regulation of immune functions in Tibetans:

We further propose to explore the mechanism of hypoxia-mediated regulation of immune functions in high-altitude adaptation of the Tibetans. It is known that the hypoxia crucially regulates the innate immune response through the HIF. It will thus be interesting to elucidate the nature of immune response in Tibetans to counter such extreme hypoxic conditions. We will screen the innate as well as adaptive immunity in Tibetans of high-altitude vs. plain-land. We will look for any association of HIF and other HIF-regulatory factors such as PHD2 and VHL with the immune responses.

Crosstalk among inflammation, hypercoagulation and thrombosis in injury-related trauma:

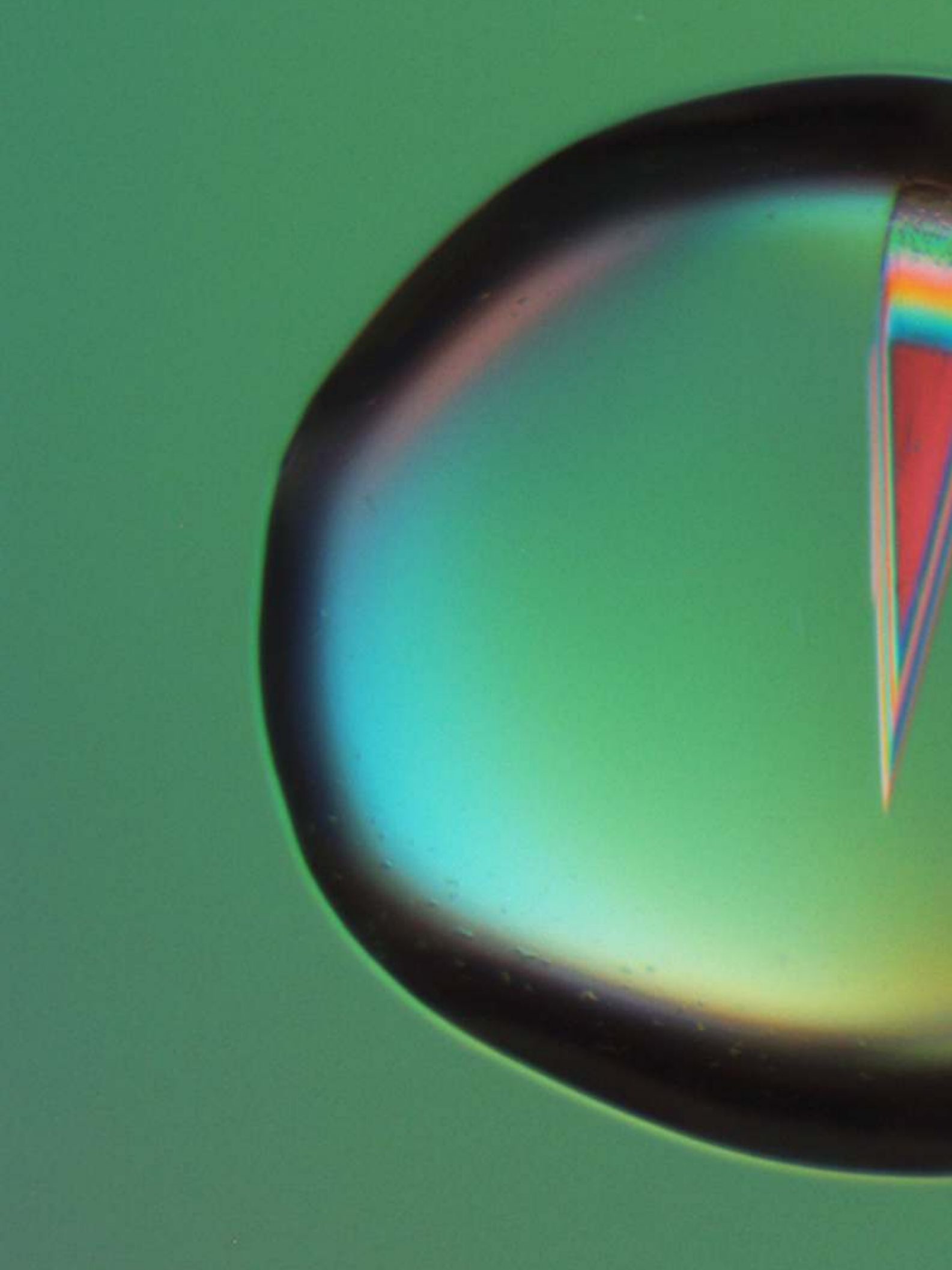
We propose to investigate the pathogenesis of acute inflammation and trauma during injury. We will determine the mechanism of crosstalk of inflammatory mediators (such as, IL and TNF) with coagulation factors (such as tissue-factor and thrombin) and pro-thrombotic factors (such as VWF, collagen, fibronectin and fibrinogen), which lead to augmented inflammation. We will also explore the role of monocytes, macrophages, platelets and their surface molecules in regulation of acute inflammation. Further we will assess the contribution of intravascular hemolysis (cell-free hemoglobin) to the pathogenesis of inflammation and trauma during injury.

Publications

Original peer reviewed articles

Lorenzo FR, Huff C, Myllymäki M, Olenchock B, Swierczek S, Tashi T, Gordeuk V, Wuran T, Li GR, McClain DL, Khan TM, Koul PA, Guchhait P, Salama ME, Xing J, Semenz GL, Liberzon E, Wilson A, Simonson TS, Jorde LB, Kaelin Jr WG, Koivunen P and Prchal JT (2014) A genetic mechanism for Tibetan high-altitude adaptation. ***Nature Genetics*** 46: 951.

Scientific Reports



New Faculty Members

Deepti Jain

Post Doc
Rockefeller University, NYC, USA

PhD 2000
National Institute of Immunology, New Delhi, India

Research Interests

The hallmark of living organism is the ability to respond appropriately to external stimuli and internal cues. These responses generally involve modulations in the gene expression profile of the organism. Gene expression in bacteria is tightly regulated at the level of transcription. In a majority of cases, regulation of transcription occurs through the use of factors that interact with DNA and/or different subunits of the RNA polymerase holoenzyme to either positively or negatively regulate the activity of the enzyme. Complete genome sequence of a number of prokaryotes has revealed the full repertoire of transcription modulators present in these organisms. However the various mechanisms utilized by these factors to regulate gene expression remain unknown even in well-studied model organisms such as Escherichia coli. The primary focus of my laboratory is to investigate the relevant molecular interactions (protein-DNA, protein-small metabolites, protein-protein) that are critical for regulating transcription in different physiological contexts. We use an integrated approach involving macromolecular crystallography, biophysical techniques, biochemical and functional assays to elucidate the functional mechanism utilized by various regulatory molecules. Currently, we aim:

- To elucidate the structural mechanism utilized by bacterial Enhancer Binding Proteins to regulate sigma54-dependent transcription initiation using FleQ from *Pseudomonas aeruginosa* as a model system.
- To understand the mechanism of transcription initiation and elongation by RpoTp – the single subunit chloroplast specific RNAP from *Arabidopsis thaliana*.
- To unearth the mechanistic basis of crosstalk between the two-component signaling circuits in *Staphylococcus aureus*.

Selected Publications

- Jain D and Nair DT (2013) Spacing between core recognition motifs determines relative orientation of AraR monomers on bipartite operators. ***Nucleic Acids Res*** 41:639
- Jain D, Kim Y, Maxwell KL, Beasley S, Zhang R, Gussin GN, Edwards A and Darst SA (2005) Crystal Structure of Bacteriophage lambda cII and its DNA complex. ***Molecular Cell*** 19:259.
- Jain D, Nickels BE, Sun L, Hochschild A and Darst SA (2004) Structure of a Ternary Transcription Activation Complex. ***Molecular Cell*** 13:45.

New Faculty Member

Deepak T Nair

Post Doc
Mount Sinai Medical Centre, USA

PhD 2001
National Institute of Immunology, New Delhi, India

Research Interests

The blue print of life for each organism is resident in its genome. Nucleic acid processing enzymes (NaPEs) play an important role in the maintenance and duplication of the genome. Perturbation in the function of these enzymes due to mutations or inhibitors has an adverse effect on the survival of the organism. We use structural, biochemical, biophysical tools coupled with functional assays to elucidate the mechanism utilized by such enzymes to achieve function. In normal circumstances, for all cellular processes to function optimally, the integrity of the genome has to be maintained. However, changes in the genome are necessary to relieve selection pressure imposed by a stressful environment. These two conflicting requirements have led to the presence of distinct pathways involving different NaPEs that either maintain genomic integrity (e.g. DNA Mismatch repair)or in adverse conditions facilitate genomic plasticity (e.g. stress induced mutagenesis). Our broad aim is to unearth the chemical and structural strategies employed by different molecular determinants of genomic integrity and plasticity to understand how these molecular throttles modulate the rate of evolution. A clear mechanistic understanding of the activity of these molecules will provide deep insight into how these molecules impact the ability of an organism to survive and propagate in diverse environments. More than 150 years ago, Darwin had postulated that new species arise through natural selection of genetic variations. Through studies on molecules that influence the appearance of these variations, we aim to contribute towards developing a more fundamental understanding of how organisms evolve and adapt.

Selected Publications

1. Surana P, Vijaya S and Nair DT (2014) RNA-dependent RNA polymerase of Japanese Encephalitis Virus binds the initiator nucleotide GTP to form a mechanistically important pre-initiation state. ***Nucleic Acids Res*** 42:2758

2. Sharma A, Kottur J, Narayanan N and Nair DT(2013) A strategically located serine residue is critical for the mutator activity of DNA Polymerase IV from Escherichia coli. ***Nucleic Acids Res*** 41:5104

3. Sharma A, Subramanian V and Nair DT(2012) The PAD region in the mycobacterial dinB homolog MsPolIV exhibits positional heterogeneity. ***Acta Crystallogr D Biol Crystallogr*** 68:960

Divya Chandran

Post Doc
University of California, Berkeley, USA

PhD 2000
University of Minnesota St.Paul, USA

Research Interests

Powdery mildews one of the most devastating fungal pathogens limiting productivity of food legumes in India and other developing countries. Current chemical methods used to control the disease are neither economical nor environmentally sustainable. Furthermore, few powdery mildew resistant legume varieties have been identified, with identity of the genes conferring resistance and knowledge of the underlying molecular mechanisms not known. Therefore, there is a need to develop novel strategies to introduce durable powdery mildew resistance in food legumes. The powdery mildew fungus is an obligate biotroph, which entirely depends on living plant tissue for survival. During pathogenesis, the fungus alters plant cellular architecture and redirects the host's metabolism via specialized structures (haustoria) to acquire nutrients while limiting host defense responses. It does so by targeting host genes and/or proteins, known as compatibility factors, specifically at the interaction site. The broad goal of my lab is to use infection site-specific analyses to identify and target novel host genes associated with defense that limit growth of the pathogen as well as host compatibility factors required for the growth and reproduction of the pathogen. We envisage that targeting a combination of such factors would result in dramatically reduced pathogen proliferation and contribute to durable resistance that is less likely to be rapidly overcome by pathogen counter-evolution. The following objectives will be pursued:

- Identification and translation of novel, infection site-specific, host molecular components controlling powdery mildew proliferation in legumes.
- Elucidation of key factors mediating carbon (re)allocation at the host-pathogen interaction site.

Selected Publications

1. Chandran D, Rickert J, Huang H, Steinwand M, Marr SK and Wildermuth MC (2014) Atypical E2F Transcriptional Repressor DEL1 Acts at the Intersection of Plant Growth and Immunity by Controlling the Hormone Salicylic Acid. ***Cell Host & Microbe*** 15:506.
2. Chandran D, Rickert J, Cherk C, Dotson B and Wildermuth MC (2013) Host cell ploidy underlying the fungal feeding site is a determinant of powdery mildew growth and reproduction. ***Mol Plant-Microbe Interact*** 26:537.
3. Chandran D, Inada N, Hather G, Kleindt CK and Wildermuth MC (2010). Laser microdissection of Arabidopsis cells at the powdery mildew infection site reveals site-specific processes and regulators. ***Proc Natl Acad Sci USA*** 107: 460.

New Faculty Member

Academic Activities and Achievements

Publications

Original peer reviewed articles

1. Bansal S, Singh M, Kidwai S, Bhargava P, Singh A, Sreekanth V, Kundu S, Singh R, and Bajaj, A (2014) Bile Acid Amphiphiles with Tunable Head Groups as Highly Selective Non-hemolytic Antitubercular Agents. ***Med Chem Commun*** (In Press).
2. Singh M, Kundu S, Reddy A, Sreekanth V, Motiani RK, Sengupta, S, Srivastava A, and Bajaj A (2014) Injectable Small Molecule Hydrogel for Localized and Sustained In Vivo Delivery of Doxorubicin. ***Nanoscale*** (In Press).
3. Singh K, Verma V, Yadav K, Sreekanth V, Kumar D, Bajaj A, and Kumar V (2014) Design, Regioselective Synthesis and Cytotoxic Evaluation of 2-Aminoimidazole-quinoline Hybrids Against Cancer and Primary Endothelial Cells. ***Eur J Med Chem*** 87C: 150.
4. Khan T and Salunke DM (2014) Adjustable locks and flexible keys: plasticity of epitope-paratope interactions in germline antibodies. ***J Immunol*** 192: 5398.
5. Gill J, Jayaswal P and Salunke DM (2014) Antigen exposure leads to rigidification of germline antibody combining site. ***J Bioinform Comput Biol*** 12: 1450006.
6. Singh M and Bajaj A (2014) Unraveling the Impact of Hydroxylation on Interactions of Bile Acid Cationic Lipids with Model Membranes by In-depth Calorimetry Studies. ***Phys Chem Chem Phys*** 16: 19266.
7. Bhargava P, Singh M, Sreekanth V and Bajaj A (2014) Nature of the Charged Head Group Determines the Fusogenic Potential and Membrane Properties of Lithocholic Acid Phospholipids. ***J Phys Chem B*** 118: 9341.
8. Kim SH, Son GH, Bhattacharjee S, Kim HJ, Nam JC, Nguyen PD, Hong JC and Gassmann W (2014) The Arabidopsis immune adaptor SRFR1 interacts with TCP transcription factors that redundantly contribute to effector-triggered immunity. ***Plant J*** 78: 978.
9. Lorenzo FR, Huff C, Myllymäki M, Olenchock B, Swierczek S, Tashi T, Gordeuk V, Wuran T, Li GR, McClain DL, Khan TM, Koul PA, Guchhait P, Salama ME, Xing J, Semenz GL, Liberzon E, Wilson A, Simonson TS, Jorde LB, Kaelin Jr WG, Koivunen P and Prchal JT (2014) A genetic mechanism for Tibetan high-altitude adaptation. ***Nature Genetics*** 46: 951.
10. Heesters BA, Das A, Chatterjee P and Carroll MC (2014) Do follicular dendritic cells regulate lupus-specific B cells? ***Mol Immunol*** 62: 283.
11. Chen C, Krishnan V, Macon K, Manne K, Narayana SV and Schneewind O (2013) Secreted Proteases Control Autolysin-mediated Biofilm Growth of Staphylococcus aureus. ***J Biol Chem*** 288: 29440.

Reviews/ Proceedings

12. Salunke DM, Gill J and Dwevedi A (2014). Comparative structural proteomics of allergenic proteins from plant pollen. ***J Ind Inst Sci*** 94: 119
13. Bhattacharjee S, Garner CM and Gassmann W (2013) New clues in the nucleus: Transcriptional reprogramming in effector-triggered immunity. ***Frontiers Plant Sci.*** 4: 364..

Lectures delivered/ Conferences attended/ Visits abroad

Dr. Dinakar M Salunke

- Delivered an invited lecture entitled "Structural Biology of Seed Proteome" in the International Conference on Plant Biotechnology, Molecular Medicine and Human Health at University of Delhi (South Campus) on 19 October 2013.
- Delivered an invited lecture entitled "New Paradigms in antibody specificity" at Centre for Biomolecular Sciences, Manipal University on 11 November 2013
- Delivered the Foundation Day Lecture entitled "New Paradigms in Immune Recognition" at National Agri-food Biotechnology Institute, Mohali on 20 February 2014
- Delivered an invited lecture entitled "Biology at the interface of Physics and Chemistry: molecules, crystals and cellular mechanisms" at Central Dept of Zoology, Tribhuvan University, Nepal on 3 April 2014
- Delivered an invited lecture on "Specificity of antigen recognition and implications of molecular mimicry" at IIT Bombay on 17 June 2014
- Co-Chaired the National Seminar on Crystallography (NSC-42) held at JNU, New Delhi during 21-23 November, 2013
- Participated in the Council of Scientific Advisors meeting of ICGEB at Trieste, Italy during 27-28 Feb 2014
- Participated in the meetings with the Nepal Academy of Science & Technology and International Centre for Integrated Mountain Development, Kathmandu, Nepal during 1-4 April, 2014
- Participated in the AAAS-TWAS workshop on Science Diplomacy at Trieste, Italy during 8-14 June, 2014
- Participated in the Global Conference on Science Advice to Governments and the 31st International Council for Science (ICSU) General Assembly at Auckland, New Zealand during 27th August – 3rd September, 2014
- Delivered an invited lecture on "Celebrating 100 years of X-ray Crystallography: A historical perspective" at Viswa-Bharati, Santiniketan on 9 October 2014
- Delivered an invited lecture on "Adjustable locks and flexible keys: Interesting insights into immune recognition" at IIT Roorkee on 8 October 2014

Dr. Prasenjit Guchhait

- EMBO-India Bioscience Young Scientist Networking Meeting, Bangalore, 7-10 November 2013

Dr. Avinash Bajaj

- Invited Talk at International Conference on Human Genetics & 39th Annual Meeting of Indian Society of Human Genetics (ISHG-2014) at Ahmedabad during 22-25 January 2014

Dr. Chittur V Srikanth

- Participated in 4th Annual Fellows meeting organized by DBT-Wellcome Trust India Alliance at Hyderabad during 1-2 October 2013
- Participated and presented seminar at in the Young scientist networking meeting at Bangalore during 7-10 November 2013

Dr. Sivaram V S Mylavarapu

- All India Cell Biology Conference on Cell Dynamics and Cell Fate, 22-24 December 2013, Bangalore India.
- Annual DBT Directors' meeting at Bhubaneswar India during 5-6 April 2014,

Dr. Tushar K Maiti

- Participated in the "Workshop on Clinical Proteomics Organized by Institute of Bioinformatics, Bangalore, during 6 – 9 January 2014
- Speaker at the 19th Training course on " Molecular Techniques as applied for infectious Diseases Research" organized by Department of Biochemistry, Mahatma Gandhi Institute of Medical Science, Sevagram, Maharashtra during 24 February – 1March 2014

Dr. Sam J Mathew

- Attended the 6th Young Investigator Meeting (YIM) held at Hyderabad from 9-13 February 2014
- Participated in the 1st AIST International Imaging Workshop at the National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan, from 19-25 January 2014.

Dr. Priyadarshini Chatterjee

- Attended workshop at Institute of Bioinformatics on Human Cell culture, Bangalore, 19-21 June 2014.

Dr. Krishnan Vengadesan

- 20th DBT's e-Library Consortium (DeLCON) Meeting held at IIT Guwahati, Assam on 28-29 October 2013.
- Participated and involved (as a local organizing committee member) in 42nd National Seminar on Crystallography and International Workshop on Applications of X-ray Diffraction in Drug Design held at JNU on 21-23 November 2013 and jointly organized by JNU, RCB, NII and AIIMS.
- Involved in coordinating "Hands on Workshop on Proteopedia" A Powerful Tool for Biomolecular Communication and a 3D Web Encyclopedia of Biomolecules – by Prof. Joel Sussman, Weizmann Institute of Science, Israel held at RCB on 30 September 2013.
- Involved in organizing workshop on Proteomics, Biomarkers and Diagnostics held at RCB on 21-25 October, 2013.

Dr. Deepti Jain

- Delivered an invited lecture at 42nd National Seminar on Crystallography and International Workshop on Application of X-ray Diffraction for Drug Discovery, at JNU, Nov. 21-23, 2013.
- Presented at International conference on Biomolecular forms and functions: A celebration of 50years of the Ramachandran Map at IISc, Bangalore. January 8-11, 2013.
- Presented at International conferenceon Biomolecular forms and functions: A celebration of 50years of the RamachandranMap at IISc, Bangalore. January 8-11, 2013.

Dr. Deepak T Nair

- Seminar titled "Mutagenic and Translesion DNA synthesis by DNA Polymerase IV from Escherichia coli : Structure and Mechanism." at Zing Conference on DNA polymerases in Biology, Diseases and Biomedical Applications held from 31/08/2014 to 4/9/2014 at Robinson College, Cambridge, UK.
- Seminar titled "Replication of the flaviviral genome: Structure of a pre-initiation state and mechanism of initiation." at the conference titled "Indo US Conference and Workshop on Recent Advances in Structural Biology & Drug Discovery" held from 9/10/2014 to 11/10/2014 at Department of Biotechnology , IIT Roorke.
- Seminar titled "Structural basis of mutagenic and translesion DNA synthesis by DNA Polymerase IV from Escherichia coli." at the conference titled "43rd National Seminar on Crystallography" held from 12/11/2014 to 14/11/2014 at Central Drug Research Institute, Lucknow.

Membership of professional / Academic bodies / Editorial Boards

Dr. Dinakar M Salunke

- Member, Governing Council, National Brain Research Centre, Manesar
- Member, Governing Body, National Institute of Plant Genome Research, New Delhi
- Member, Governing Body, Translational Health Science & Technology Institute, Gurgaon
- Independent Director, Biotechnology Industry Research Assistance Council (BIRAC) Board
- Member, Commission on Biological Macromolecules, International Union of Crystallography
- Vice President, Indian National Science Academy
- Member, Scientific Advisory Board, UNESCO-International Institute of Biotechnology, Nigeria
- Member, Council of Scientific Advisers, International Centre for Genetic Engineering & Biotechnology
- Member, Scientific Advisory Committee, National Brain Research Centre, Manesar
- Member, Scientific Advisory Committee for Biosciences and Bioengineering Group, IIT Indore
- Chairman, INSA National Committee for International Union of Crystallography
- Member of Expert Committee, Fund for Improvement of S&T Infrastructure in Higher Educational Institutions (FIST), Department of Science & Technology
- Member, Apex Committee, Biotechnology Industry Partnership Programme
- Member, Finance Committee, National Brain Research Centre, Manesar
- Member, INSA Sectional Committee on Cell and Biomolecular Sciences
- Member, Committee of Experts for Planning Synchrotron Radiation Sources, Office of the PSA, Govt of India
- Member, Research Council, CSIR-IGIB, Delhi
- Member, Research Council, CSIR-CCMB, Delhi
- Chairman, Audit Committee, Biotechnology Industry Research Assistance Council
- Member, First Court of Tripura University

Dr. Prasenjit Guchhait

- Member, Editorial Board, Journal of Hypertension and Cardiology
- Member, Editorial Board, World Journal for Hypertension

Dr. Chittur V Srikanth

- Member, Task Force in Infectious Disease Biology, Department of Biotechnology , Govt of India
- Member, Editorial Board, Austin Journal of Biotechnology and Bioengineering

Dr. Tushar K Maiti

- Member, Task Force in Human Development and Disease Biology, Department of Biotechnology , Govt of India
- Member, Editorial Board, Austin Journal of Biotechnology and Bioengineering

Dr. Sivaram V S Mylavarapu

- Member, Human Ethics Committee, THSTI, Gurgaon

Distinctions, Honours and Awards

1. Dr. Dinakar M Salunke - Elected Fellow, The World Academy of Sciences
2. Dr. Dinakar M Salunke - JC Bose National Fellow, DST
3. Dr. C V Srikanth - Wellcome Trust/ DBT India Alliance Intermediate Fellow
4. Dr. Sam J Mathew - Wellcome Trust/ DBT India Alliance Intermediate Fellow
5. Dr. Puspha Kumari - Welcome Trust/ DBT India Alliance Early Career Fellow
6. Dr. Avinash Bajaj - Ramanujan Fellow, DST
7. Dr. Saikat Bhattacharjee - Ramalingaswami Fellow, DBT
8. Dr. Deepti Jain - Innovative Young Biotechnologist Awardee, DBT
9. Mr. Sagar Mahalle - Won the Gold Prize for the best presentation at the 1st AIST International Imaging Workshop held at AIST, Tsukuba, Japan, from January 20-24, 2014

Foundation Day Lecture

In commemoration of RCB Foundation Day, the Foundation Day Lecture has been instituted. This year, Dr. R Chidambaram, Principal Scientific Advisor to the Govt. of India, delivered lecture entitled "Internationalization of Science and the Knowledge Economy", on 19th June 2014. Dr. Chidambaram emphasized that India may be developing country in a broader sense, but it is much developed in the scientific arena. He reviewed scientific developments in India in a wide range of fields and highlighted, with examples, the need for international collaborations in the context of developing knowledge-based economy.

Academic Activities and Achievements

RCB Colloquia

Coinciding with the meetings of the Programme Advisory Committee (PAC) of the Centre, Scientific Colloquium Lectures are delivered by outstanding scientists from reputed institutions across the globe. During the last PAC meeting Colloquium lectures were delivered by Dr Satyajit Rath, NII, New Delhi and Prof. K. Veluthambi, Madurai Kamaraj University, Madurai.

Dr. Satyajit Rath delivered an interesting lecture on 'Misleading randomness, antifragility and the Red Queen problem: case studies in immunological heterogeneity'. He presented as-yet-unpublished data from a couple of areas to discuss the sources and consequences of immune heterogeneity, ranging from studies in induced heterogeneity hidden in apparently unimodally distributed stochastic variation in T lymphocytes, through the role of T cell population heterogeneity as a determinant of dose-response relationships, to field measurements of human immune heterogeneity that provide interesting insights into both developmental and functional inter-individual variations.

Prof. Veluthambi talked about 'Rice functional genomics through Agrobacterium T-DNA tagging'. He made a detailed presentation on research findings on the work related to Genetic engineering of male sterile rice. The audience interestingly noted that The T-DNA-tagged rice mutant TC-19, which harboured a single copy of the T-DNA, displayed male sterile phenotype in the homozygous condition and female fertility was normal in the TC-19 mutant. Analysis of the junctions between the T-DNA ends and the rice genome revealed a complex integration in which the T-DNA, together with a 6.55-kb region of chromosome 3 and a 29.8-kb region of chromosome 9, was translocated into chromosome 8. The presentation highlighted the crucial role of the T-DNA-tagged Os08g0152500 gene in stamen development in rice and the promoter of this gene holds promise to engineer 'heterosis' in rice.

Academic Activities and Achievements

Distinguished Lecture Series

With the objective of creating an international platform for discussions and debates in a range of Biotechnology research areas, leading specialist and eminent scientists from the national and international institutions are invited to deliver lectures and share thoughts, ideas and research methodologies. The focus has been to highlight research challenges and solutions in their field of research. Following eminent scientists delivered distinguished lectures during this year:

1. Activation-Triggered Subunit Exchange in the Dodecameric Calcium/Calmodulin-Dependent Protein Kinase Holoenzyme by Prof. John Kuriyan, Department of Chemistry, University of California, Berkeley

2. Controlling Brain and Brawn Development: Regulating Stem Cell Expansion and Lineage Properties by Prof. K VijayRaghavan, Secretary, DBT, Government of India & Senior Professor, NCBS, Bangalore

3. Social Regulation of Reproduction in an Insect Society by Prof. Raghavendra Gadagkar, President, INSA & INSA S N Bose Research Professor, Centre for Ecological Sciences, Indian Institute of Science, Bangalore

4. Studies of Assembly and Maturation of ds DNA Viruses Reveal Novel Mechanisms Driving Biological Dynamics by Prof. Jack Johnson, The Scripps Research Institute, La Jolla, California

Academic Activities and Achievements

Education, Research and Training Activities

Multidisciplinary PhD Programmes

Multidisciplinary doctoral programme has been instituted for students who have completed Masters in the relevant disciplines of natural sciences, medicine, engineering and related fields of study. Candidates who have obtained M.Sc degree in any field of Science (or equivalent) having intense interest to work at the interface of multiple disciplines in the areas related (but not limited) to structural, systems, synthetic and chemical biology, tissue engineering, analysis of complex diseases for identification of intervention points and development of knowledge-based drug discovery strategies, are enrolled as Junior Research Fellows (JRFs) to work under the mentorship of the faculty. The fellowship is initially tenable for the duration of three years and is extendable for additional two years after a review. JRFs are recruited twice during the academic year. As on the current academic year, more than 50 students are enrolled as JRF/ SRFs leading to the PhD programme.

Young Investigator (YI) & Post Doctoral Fellowship Awards Programme

YI Awards scheme has been initiated to nurture outstanding recent PhD fellows with the aim to pursue novel discoveries under the mentorship of the RCB Faculty. Under this programme, the Centre enrols young scientists from within India and outside in this region to carry out research in various cross-cutting areas of biotechnology. During the year, the Young Investigator programme has been augmented and suitable candidates have been engaged. In addition, a few post-doc fellows are also being mentored by the RCB faculty.

Short-term Training

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

In addition, short-term training programme in platform technologies towards skills development in multiple areas such as Cell & Tissue engineering, Nano-biosciences, biomedical engineering, climate sciences and energy resource management are planned.

Master's Degree in Medical Science

RCB is in the processes of designing a flagship Master's degree programme in Medical Sciences. The programme is being designed with the objective of providing knowledge in life sciences with emphasis on human biology, clinical and translational research. The

programme will be offered deriving expertise from within RCB as well as from other national institutions including National Institute of Immunology (NII), Translational Health Science and Technology Institute (THSTI), National Institute of Biomedical Genomics (NIBMG), among others. The overall aim of this course shall be to build the aspects of practical learning and research skills for medical and related graduates that will enhance educational experience and contribute to making of competent medical researcher.

The Centre is at the early stages of conceptualizing Integrated Masters' programme in Biotechnology and leading to award of PhD.

Status of RCB Bill

The Regional Centre for Biotechnology Bill 2014 is designed to enact a law for declaring the RCB as an institution of national importance, so that the centre is empowered to undertake human resource development programmes in the field of biotechnology and award degrees in these and related disciplines. Earlier, RCB Bill 2011 was presented to the Lok Sabha. After due process, the Bill was listed in the schedule of business of the 15th Lok Sabha. However, the Lok Sabha could not pass the Bill as per the schedule of business. Steps are being taken for placing the RCB Bill 2014 in the 17th Lok Sabha.

Workshops & Study Visits

International Workshops and Advanced School

At RCB, educational and training activities have been initiated in the right earnest. Several workshops and advanced school programme in cross-cutting areas of biotechnology were conducted for strengthening the academic purview of this centre and also to boost knowledge potential. During the reporting period, the following workshops are being organized in RCB: Towards achieving excellence in training and education an Advanced School of Training is being conducted in 'Diabetics and Metabolic Syndrome'. This is being organised in partnership with IUBMB & FAOPMB. The training will be to open all the researchers in biology & medical sciences with focus on diabetes and metabolic disorders.

The Centre is organizing an International Workshop-cum-symposium on recent advances in frontier areas of structural biology focussing on cryo-electron microscopy and free electron lasers in Dec 2014 in partnership with Indian National Science Academy (INSA) where all scientists, researchers and mid-career scientists from all Asian countries will participate.

With the support of UNESCO, RCB will be holding a 'Regional Dialogue on Science & Technology Policy' where in eminent scientists, policy makers, researchers, heads of universities, academicians from Asian countries will take part. This will be one of unique event where they will be sharing their knowledge, development in S&T in their countries, industrial development and present status of education and research.

Post-graduate students of Biotechnology from the University of Mysore were given orientation to biotechnology research. As part of the short-term training programme, graduate and post-graduate students of science, engineering from various colleges, universities carry out project, dissertation work towards partial fulfilment of their programmes of study.

Seminars delivered by Visiting Scientists

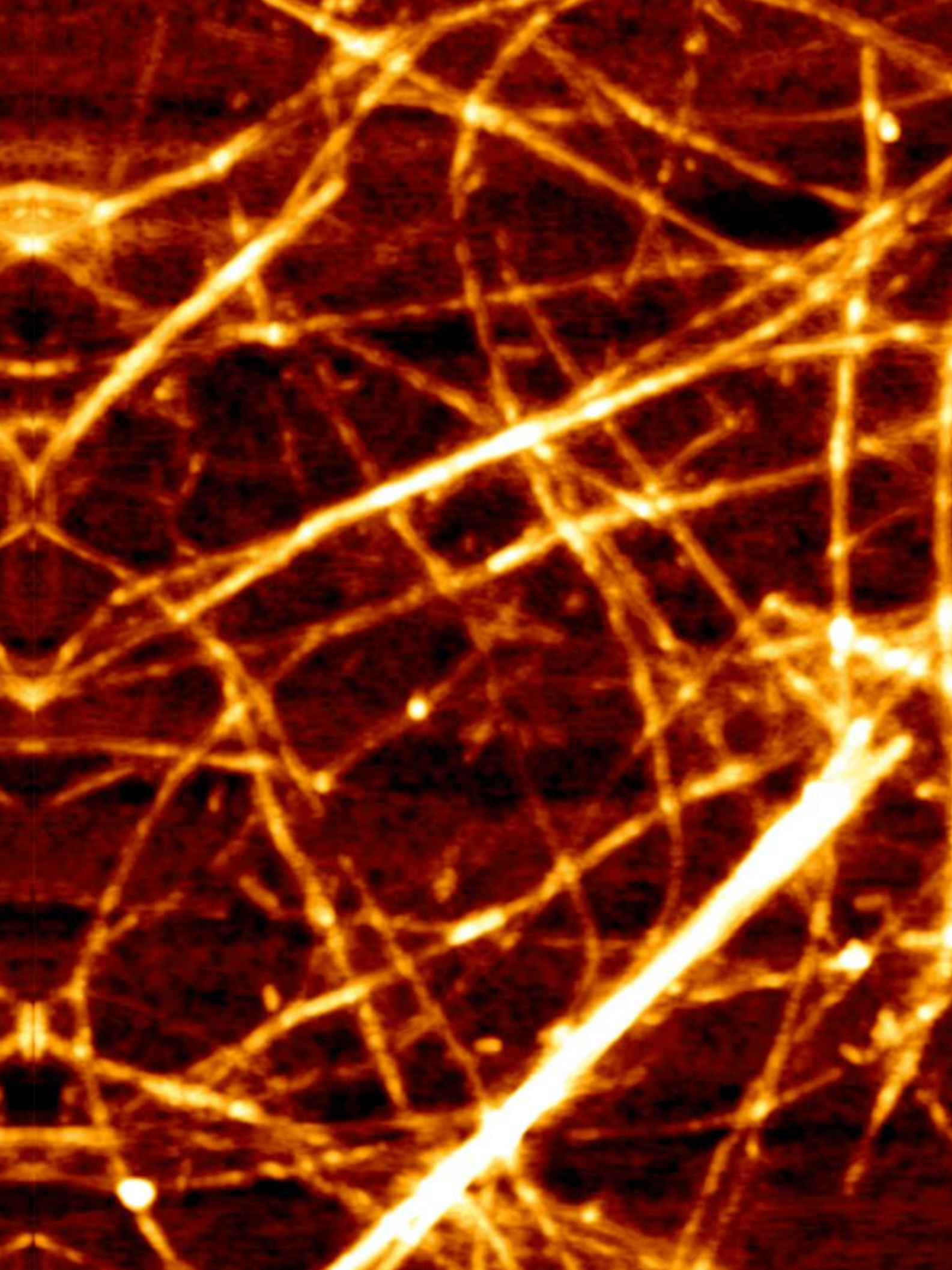
Speaker	Title	Date
Usha Vijayraghavan, PhD Prof. Indian Institute of Science, Bangalore	Functions for some evolutionarily conserved transcription regulators in rice inflorescence and flower development	September 15, 2014
Debabrata Dash, MD, PhD Dept. of Biochemistry, Institute of Medical Sciences, Banaras Hindu University	Playing with Platelets	September 08, 2014
Apurva Sarin, PhD National Centre for Biological Science, Bangalore	Notch signaling integrates pathways controlling cellular homeostasis	September 05, 2014
Rajendra P Roy, PhD Senior Scientist, National Institute of Immunology, Delhi	Sortase: A marvelous enzymatic tool for protein engineering	August 12, 2014
Ramesh V Sonti, PhD Chief Scientist, CSIR-Centre for Cellular and Molecular Biology, Hyderabad	Induction and Suppression of Damp Induced Innate Immunity in Rice-Bacterial Interactions	August 08, 2014
B Gopal, PhD Indian Institute of Science, Bangalore	Structural insights into the diverse mechanisms that govern cell wall synthesis in Staphylococcus aureus	August 04, 2014
Vaibhav Agarwal. PhD Lund University, Sweden	Understanding the role of complement system in Streptococcus pneumoniae-host interactions and its strategies of complement evasion	July 25, 2014
Prof. D N Rao Indian Institute of Science, Bangalore	Natural Transformation vis-a-vis Restriction-Modification Systems in Helicobacter pylori	July 21, 2014
Dr John Wotherspoon Regional Marketing Director, Asia Pacific, BD Biosciences	Multicolour Panel Design Tools	June 26, 2014
Vidisha Tripathi, PhD University of Illinois, USA	Mammalian long Noncoding RNAs: Regulators of Gene Expression	May 01, 2014
Vasanta Subramanian, PhD, FSB Department of Biology and Biochemistry, University of Bath, Bath, United Kingdom	The Role of the Primary Cilia in Sensing Differentiation Cues and in Cerebellar Patterning- Insights from Targeted Mouse Mutants and Embryonic Stem Cell Models	Apr 24, 2014
Arvind Sahu, PhD National Centre for Cell Science Pune, India	Virally encoded complement regulators as tools to understand human complement regulation	Apr 16, 2014
Donald McClain, M.D. Ph.D Professor of Medicine (Endocrinology and Metabolism), University of Utah, Salt Lake City, Utah, USA.	Hypoxia, Iron, and Diabtetes.	Apr 04, 2014

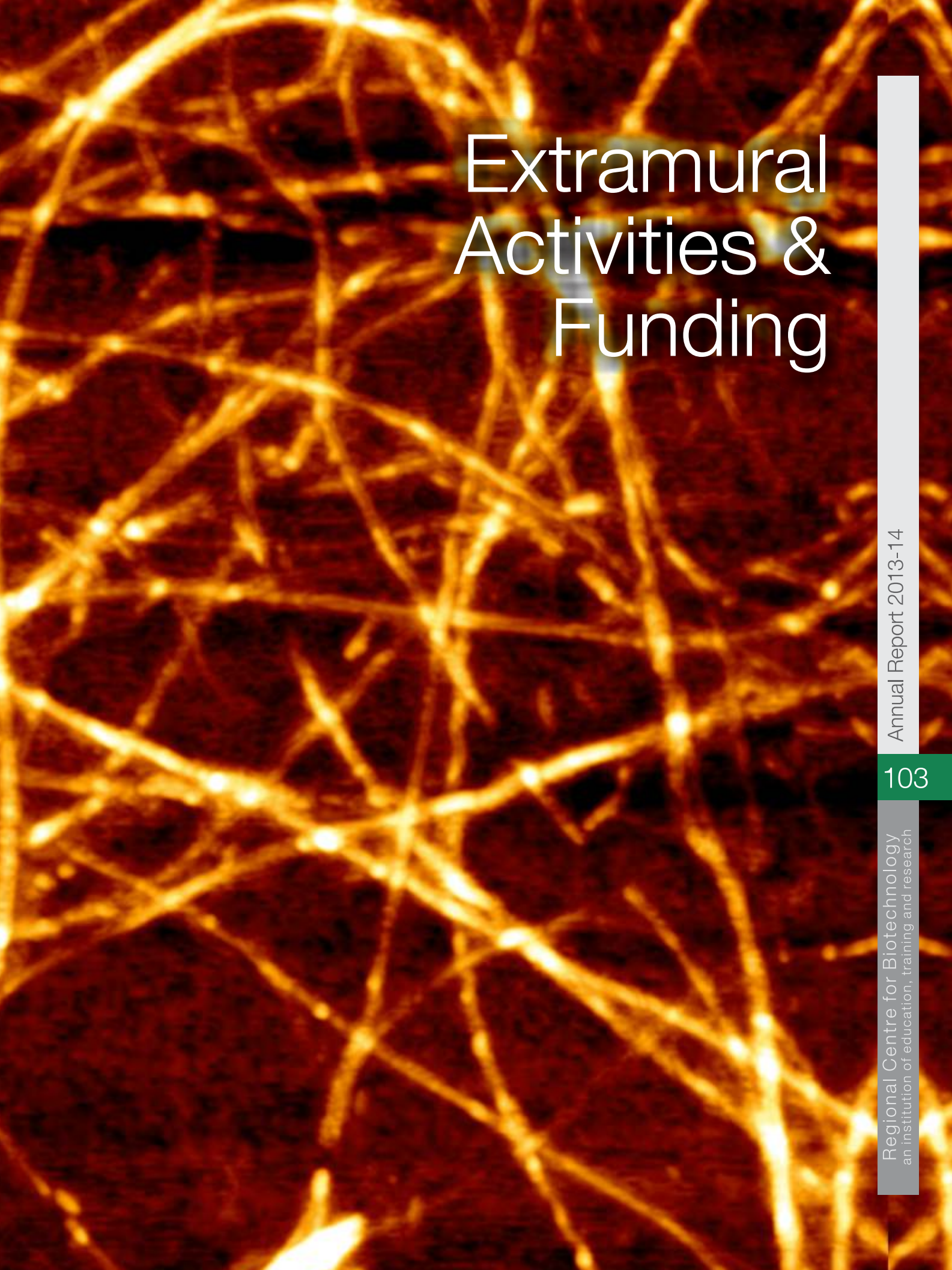
Speaker	Title	Date
Josef T Prchal, M.D. Professor of Medicine (Hematology), Pathology and Genetics,University of Utah, Salt Lake City, Utah, USA.	Interaction of Germline and Somatic Mutations in Polycythemia Vera, HIFs and Tibetan Hypoxic Adaptation.	Apr 01, 2014
Naresh C Bal, PhD Department of Physiology and Cell Biology The Ohio State University, USA	Understanding the role of skeletal muscle bey Assessing India's wildlife health and conservation status- a comprehensive approach ond contraction	Mar 20, 2014
Samrat Mondol, PhD University of Seattle, USA	Assessing India's wildlife health and conservation status- a comprehensive approach	Mar 07, 2014
Prem Kumar Sinha, PhD The Pennsylvania State University, Hershey, USA	Structural and molecular mechanism of energy transduction in the bacterial respiratory complex I (NDH-1)	Mar 07, 2014
Amit Prasad, PhD Division of Rheumatology, Allergy & Immunology Center for Immunology and Inflammatory Diseases, Charlestown, MA	Inflammation: friend or foe, Role of innate immune cells in infectious and auto-immune disorders	Mar 06, 2014
Nixon M Abraham, PhD University Medical Centre, University of Geneva,Geneva, Switzerland	Sniffing to decide: temporal aspects of olfactory information processing.	Feb 28, 2014
Gyanendra P Dubedy, PhD Department of Microbiology and Molecular Genetics The Hebrew University of Jerusalem, Israel	Characteristics and Dynamics of Bacterial Nanotubes	Feb 25, 2014
Balananda Dhurjati Kumar Putcha, PhD University of Alabama at Birmingham, USA	Allosteric Regulation of Thyroid Receptor Activation	Feb 25, 2014
Gopinath Meenakshisundaram, PhD Institute of Medical biology, Singapore	Non-coding and coding function of a single mRNA dictates skin homeostasis in wound healing and cancer	Feb 24, 2014
Richa Jaiswal, PhD Department of Biology, Brandeis University, Waltham, MA, USA.	New collaborations of formins in actin assembly and organization	Feb 17, 2014
Jagadis Gupta Kapuganti, PhD Marie Curie Fellow, Biochemistry & Systems Biology Department Of Plant Sciences University Of Oxford UK	Nitric oxide in plants: the biosynthesis and cell signalling functions of a fascinating molecule in plants	Feb 14, 2014
Vinothkumar KR, PhD MRC-Laboratory of Molecular Biology, Cambridge	Architecture of mammalian mitochondrial respiratory complex I	Feb 11, 2014
Sachin Deshmukh, PhD Mind/Brain Institute, Johns Hopkins University ,Baltimore, MD, USA	Objects, space, and memory: how the hippocampal cognitive map comes together	Feb 6, 2014

Speaker	Title	Date
N Sadananda Singh, PhD Tamasek Lifesciences Lab, NUS Singapore	Understanding cytokinesis: lessons from fission yeast study	Jan 31, 2014
Partha Ray, PhD Department of Surgery Duke University Medical Center, Durham, North Carolina, USA	Application of Aptamers for Therapeutics	Jan 30, 2014
Anurag Agrawal, PhD Institute of Genomics and Integrative Biology (IGIB) New Delhi.	Making a three-way marriage work: A story of Maths, Biology, and Medicine	Jan 9, 2014
Areejit Samal, PhD Abdus Salam International Centre for Theoretical Physics Trieste, Italy	Reconstruction and systems analysis of plant cell wall deconstruction network in filamentous fungus Neurospora crassa	Jan 8, 2014
Diptiman Chanda Department of Pathology University of Alabama Birmingham, USA	Gene And Cell Based Therapies For Metastatic Cancer	Dec 18, 2013
Carmen Gervais, PhD Director of Fellowships and Career Development	Living on the edge: scientific careers at the frontiers of research	Dec 06, 2013
Fernando Vargas, PhD Senior Application Scientist WITec GmbH, Germany	Applications of Confocal Raman Microscopy and allied modules in biological and life science researches.	Dec 05, 2013
Shyam K Masakapalli, PhD Department of Biology and Biochemistry, University of Bath, UK	Mapping cellular metabolism with fluxomics	Dec 04, 2013
Rashmi Gupta, PhD Max Planck Institute for Infection Biology, GERMANY	The transport mechanism for Salmonella typhimurium Type 3 Secretion System SPI-1 is influenced by the needle channel properties	Dec 04, 2013
Hiyaa Singhee Ghosh, PhD Columbia University Medical Center New York, USA	Plasmacytoid Dendritic Cell lineage specification and maintenance: a story of E, ETO and ID proteins.	Nov 19, 2013
Amit Kumar, PhD FIRC Institute of Molecular Oncolgy Foundation Milan ,Italy	An ATR-mediated mechanica l response controlling cell plasticity	Nov 1, 2013
Rajesh Patkar, PhD Temasek Life Science Lab, NUS Singapore	The ABC of Small Molecule Transport in Fungal Pathogenesis	Oct 28, 2013
Sreeramaiah Gangappa, PhD National Institute of Technology, Durgapur West Bengal, India	"Role of BBX25, a novel HY5 interacting B-BOX protein, in Arabidopsis seedling photomorphogenesis"	Oct 28, 2013
T P Singh, PhD, AIIMS New Delhi	Protein-3D structure and drug discovery	Oct 25, 2013

Speaker	Title	Date
Nagasuma Chandra, PhD IISc, Bangalore	Systems biology and protein interaction network	Oct 25, 2013
Rajesh S Gokhale, PhD IGIB, New Delhi	Deciphering chemico-cellular trestle of mycobacteria to elucidate disease physiology	Oct 25, 2013
Deepak T Nair, PhD NCBS, Bangalore	Structural proteomics: Present and future	Oct 25, 2013
Shobhalata Udapudi, PhD GNLU, Gandhinagar	Patent aspects for biotechnology inventions	Oct 24, 2013
Sanjeeva Srivastava, PhD IIT, Mumbai	Proteomics and Diseases	Oct 24, 2013
Abhijit De, PhD ACTREC, Mumbai	Newer techniques in biomolecular imaging	Oct 23, 2013
Pravat K Mandal, PhD NBRC, Manesar	Imaging and biomarkers	Oct 23, 2013
Debasis Mohanty, PhD NII, New Delhi	Proteomics and bioinformatics	Oct 22, 2013
Suman Thakur, PhD CCMB, Hyderabad	Mass spectrometry, proteomics & protein-protein interactions	Oct 22, 2013
Prasanna Venkatraman, PhD ACTREC, Mumbai	Proteomics and its implications in biology	Oct 22, 2013
Mahesh J Kulkarni, PhD NCL, Pune	Clinical proteomics - Mass spectrometric analysis of protein modifications	Oct 21, 2013
Rapole Srikanth, PhD NCCS, Pune	Proteomics data analysis and statistical validation	Oct 21, 2013
Ramesh Ummanni, PhD IICT, Hyderabad	Proteomics and biomarkers	Oct 21, 2013
Deepak T Nair, PhD National Centre for Biological Sciences Bangalore, India	Structural basis of mutagenic and translesion DNA synthesis by DNA Polymerase IV from Escherichia coli	Oct 11, 2013
Anupam Paliwal, PhD Institut Pasteur Paris, France	DNA methylation in cancer and other complex-diseases	Oct 9, 2013

Academic Activities and Achievements





Extramural Activities & Funding

Extramural Activities & Funding

Multi-institutional Preterm Birth Programme (PTB)

A multi-institutional study, of which RCB is an important partner, on pre-term birth involving a very large cohort ha sbeen initiated. The basic aim of the project is to understand the epidemiology of PTB, its genetic and environmental interactions, and changes in vaginal microbial landscape. The program highlights include development and evaluation of putative biomarkers, identification of simple microbiological tool based vaginal risk factors, modulation of vaginal microbiota for therapeutic purposes and evaluation of environmental modification chosen from SNP analysis. Some of the major public health concerns addressed by the program are biological risks and processes of fetal growth and PTB, clinical consequences of PTB and intra uterine growth retardation.

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

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

The team also comprises of a clinical team and a project management team. The clinical team will be stationed at Gurgaon General Hospital, which is the site of study, and comprises of the clinical coordinator, research physicians, nurses, attendants, field workers and field supervisors.

RCB's primary role is to address the proteomics-based questions. Proteomic analysis will provide the differentially regulated proteins and protein function alteration due to posttranslational modification of protein in the preterm birth condition. Ultimate goal of the RCB's approach is to decipher the molecular machanisms contributing to the poor pregnancy outcome, based on proteomics approach.

Biotech Science Cluster, Faridabad

RCB is an important component in the Biotech Science Cluster at Faridabad (BSCF) being set up in Faridabad, NCR of Delhi. The Biotech Science cluster is envisaged to support innovation research, facilitate public-private partnership for the development of biotechnology business incubators and parks, including creative partnerships with Biotech & Pharma entrepreneurs and distributors. The cluster will network the potential of its constituent institutions to create a comprehensive ecosystem for accelerating discoveries and facilitating translation of these discoveries in real world solutions in the field of health care and agriculture and their commercialization. In terms of infrastructure, the cluster will have a state-of-art technology platform center, animal facilities and biosafety containment laboratories.

Advanced Technology Platform Centre

RCB plays a key role in the setting up of Advanced Technology Platform Centre, which would act as a catalyst for multidisciplinary basic and translational research and development by providing relevant state of the art instrumentation, training and professional services for the stakeholders and others alike on behalf of Biotech Science Cluster in Faridabad. The Advanced Technology Platform Centre (ATPC) is an initiative for multidisciplinary research that translates scientific and technological advancements into innovations that will improve public health.

Bio-Incubator

The technology business Bio-incubator being established for BSCF, in partnership with BIRAC, would provide new and emerging technology with compatible environment that would support their start-up phase and increase their likelihood of success, cater to the needs of companies acquiring technology from abroad for soft landing also provide facilities for pilot scale lot production under GMP for new products also facilitate prototype to product conversion for devices and implants. The planned incubator includes facility space, flexible leases, shared use of common office.

International Networking

AIST, Japan

The Regional Centre for Biotechnology (RCB) and the National Institute of Advanced Industrial Science & Technology (AIST), Japan announced a new partnership to further capacity building initiatives in bioimaging and biotechnology. The Memorandum of Agreement (MoA) was signed on March 4, 2014. The initiative will enhance career opportunities for scientists and researchers working in biomedical, clinical, and other related areas of biotechnology and complement the existing bilateral research cooperation between the Govt. of India and the Govt. of Japan.

The Memorandum of Agreement will enable RCB and AIST to jointly organize advanced research training in bio-imaging and biotechnology, including in vivo and in vitro imaging, cryo-electron microscopy crystallography and such other technologies. Key features of the agreement are: to set up a DBT-AIST International Laboratory for Advanced Bio-imaging

RCB students participating in training at AIST, Japan

Extramural Activities & Funding

(DAILAB) at RCB and to facilitate joint collaborations engaging Indian and Japanese scientists and to support selected Indian researchers for training in specialized areas of bio-imaging and biotechnology at AIST as well as other laboratories outside AIST in Japan.

The new collaboration provides an opportunity for both the institutions in capacity building, training and research collaborations and will benefit young scientists not only in India and Japan but also from the UNESCO member countries in the Asia-Pacific and SAARC regions. Indeed, through the current initiative, 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 betterment of mankind.

IUBMB

Advanced training on biotechnology specialised areas is part of IUBMB. They have funded RCB to impart training in Diabetic and Metabolic Syndrome to national and international participants who are young scholars/ researchers, PhD students and mid-career scientists. The workshop will have faculty derived from within India and global universities/ institutes to India to impart this training. This is the first of its kind in the new biotechnology areas and specifically for the Asia Pacific region.

Other Programmes

Technology Advancement Unit

Technology Advancement Unit (TAU) is a joint initiative of the Swiss Agency for Development and Collaboration and the Department of Biotechnology. The Technology Advancement Unit is being set up with the aim to create a conducive environment for the planning and implementation of R&D projects, focussed on product development/ technology transfer thereby promoting technology advancement and transfer in the development context. RCB is managing the Technology Advancement Unit. As part of its multi-dimensional role of an inter-institutional coordinator, RCB has hosted the TAU and has provided administrative facilities for TAU.

Biosafety Support Unit

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

Extramural Activities & Funding

Extramural Project Funding

1. Understanding Salmonella-mediated alterations in host SUMOylation: implications in infection and inflammation: Dr. C.V. Srikanth; Rs. 328 Lakhs; (Wellcome Trust/ DBT India Alliance Intermediate Fellowship)

2. Role of developmental myosin heavy chains in skeletal muscle development, regeneration, homeostasis and disease: Dr. Sam J Mathew, Rs. 350 Lakhs; (Wellcome Trust/ DBT India Alliance Intermediate Fellowship)
3. Understanding role of Exocyst complex in cell division and development in Caenorhabditis elegans: Dr. Puspha Kumari, Rs. 144 Lakhs; (Welcome Trust/ DBT India Alliance Early Career Fellowship)
4. Engineering of Nanomaterials and their interactions with DNA and Cell Surface: Dr. Avinash Bajaj, Rs. 73.00 lakhs; (Ramanujan Fellowship, DST)
5. Elucidating inositol-dependent signalling routes of effector-triggered immunity for identifying new approaches for engineering crop resistance against diverse pathogens: Dr. Saikat Bhattacharjee, Rs. 82.00 lakhs; (Ramalingaswami Fellowship, DBT)
6. Structure and mechanism of FleQ, master regulation of transcription of flagellar and biofilm genes in Pseudomonas aeruginosa: Dr. Deepti Jain, Rs. 52 Lakhs (Innovative Young Biotechnologist Awardee, DBT)
7. Inter-institutional programme for Maternal, Neonatal and Infant sciences a Translational approach to studying PTB: Dr. Dinakar Salunke and Dr. Tushar K Maiti, Rs. 48.85 Cr. Jointly with THSTI, NIBMG and General Hospital, Gurgaon, NII, and Safdarjung Hospital; (DBT)
8. Engineering of Nanomaterials for Combination Cancer Therapy: Dr. Avinash Bajaj, Rs. 30.00 lakhs; (DBT)
9. Collaboration for translation and clinical research between Translation Health Science and Technology Institute, National Brain Research Centre, Regional Centre for Biotechnology and Gurgaon, Civil Hospital: Dr. Dinakar M Salunke, Rs.79.05 lakhs; (DBT)
10. Pathophysiology of thrombocytopenia in dengue infection: Dr. Prasenjit Guchhait, Rs. 55 lakhs, Jointly with AIIMS, New Delhi; (DBT)
11. Molecular Basis for Silencing of the Spindle Assembly Checkpoint: Dr. Sivaram Mylavarapu, Rs. 36 lakhs; (DBT)
12. Phospholipid based Nanomaterials as Novel Therapeutics for Cancer: Dr. Avinash Bajaj, Rs. 29.0 lakhs; (DBT)
13. Structural investigations of surface nano scale assembly in a gut bacterium: Dr K Vengadesan, ~60 Lakhs; (Being processed by DBT).
14. Design, Engineering, and Investigating the Anticancer/anti-angiogenic Properties of Bile Acid Amphiphiles for Colon Cancer Therapy: Dr. Avinash Bajaj, ~Rs 60 lakhs Jointly with NII, New Delhi (Being processed by DBT).
15. The role of MET-CBL signaling in Rhabdomyosarcoma: Dr. Sam J Mathew, ~Rs 25 lakhs; (Being processed by DBT).
16. Targeting ubiquitin proteasome system for the anticancer drug development: A peptoid based inhibitor design, synthesis and evaluation: Dr. Tushar K Maiti, ~Rs 25 lakhs; (Being processed by DBT).

Infrastructure & Development

Administrative Activities

During this year, the position of staff in the Centre was enhanced to keep up with the growing needs of the Academic, Research and Technical activities by recruitment of staff with expertise in areas of Finance, Administration and Stores & Purchase on contractual basis. The processes & procedures for conduct of the administrative, financial, engineering and inventory management activities at the Centre were undertaken seamlessly to enhance the functioning of the centre. It has also provided adequate service support to establishment of the Advanced Technology Platform Centre and the Bio-Incubator, which are two of the important activity Centres of the Biotech Science Cluster in Faridabad and the Technology Advancement Unit and Biosafety Support Unit which are the initiatives of the Department of Biotechnology, Govt of India.

Library & e-library facility has been fully established at the RCB with regular subscription of both hardcopy journals as well as e-journals. The access to a wide range of e-journals provided by the consortium of DELCON is also available in the library. RCB, being part of the National Knowledge Network, has benefitted hugely by the availability of the large bandwidth. Further up-gradation of the IT facilities is being constantly undertaken, with active cooperation & guidance of the Officers of the NIC cell in DBT.

The Centre has been functioning in conformity with the guidelines of the Government of India with regards to reservation of posts, programmes for implementation of official language and other activities.

Laboratory Infrastructure

The Interim Laboratories at 180, Udyog Vihar, Phase I, Gurgaon (NCR were furnished with equipments, instruments and facilities like Nuclear Magnetic Resonance Spectrometer (NMR), Advanced Proteomics Facility (Mass Spectrometry), Surface Plasmon Resonance, Atomic Force Microscope(BIO-AFM), Protein/Peptide Sequencer, FPLC protein purification system, Dispenser 4 Crystallisation, CD Spectro polarimeter, Flow Cytometer, Workstations (8Nos.), Isothermal Titration Calorimeter, Floor Ultra Centrifuge, Fluroscence Microscope, Differential Scanning Calorimeter, CCD imager, Stackable incubator shaker, Shakerincubator,-80 Deep Freezer, High capacity Refrigerated Centrifuge, Real Time PCR machine, Automated flash chromatography system, Weighing Balance, HPLC accessories, Microbial Cell Disrupter, Stereo Microscope, Vibration Free Cooled Incubators, Evaporative light scattering detec., Tissue Homogenizer, Cold Rooms, Refrigerated Centrifuge, Refrigerated Centrifuge, MCT Detector and Nano Spectrophotometer. A Seminar Hall with state of art facilities has been established which can accommodate over 150 participants. The recently established seminar hall will cater to the requirement of RCB and also sister institutes in the vicinity. This is in addition to the regular Class room facility and conference room, which are already equipped with state-of-art facilities including the Video Conferencing facility.

Permanent Campus of the Biotech Science Cluster at Faridabad

The permanent Campus of RCB as part of the NCR-BSC Project at Faridabad, initiated as Phase-I of construction, consisting of the Laboratory buildings, is now ready for occupation. With the process of installation of supporting infrastructural services and furnishing of the labs being undertaken, the permanent campus of RCB is expected to be operational very soon. The timely initiation of the construction activity and close monitoring of the progress of work has resulted in successfully setting up of the infrastructure of the Bio-tech Science Cluster at Faridabad.

Simultaneously, the construction activity for the 4th Wing of the RCB Laboratory, hostels for students and housing for faculty has also commended as part of the Phase - I (Extension). Since RCB has also been entrusted with the task of setting up the Advanced Technology Platform Centre & the Bio-incubator, the construction of the building to cater to the requirements of these two centres have also been initiated.

Institutional Information

Board of Governors

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

Programme Advisory Committee

1.	Prof. Angelo Azzi Tufts University 711 Washington ST. Boston MA 02111, USA	Chairperson
2.	Prof. Subrata Sinha Director National Brain Research Centre NH8, Nainwal Mode Manesar - 122051	Member
3.	Dr. Satyajit Rath Senior Scientist National Institute of Immunology Aruna Asaf Ali Marg, JNU Campus New Dellhi-110067	Member
4.	Prof. K Veluthambi School of Biotechnology, Madurai Kamaraj University Palkalai Nagar Madurai-625021	Member
5.	Mr. S Sinha Advisor Department of Biotechnology MoS&T, Govt. of India New Delhi-110003	Member
6.	Dr. K V S Rao International Centre for Genetic Engineering and Biotechnology, ICGEB Campus Aruna Asaf Ali Marg New Delhi-110067	Member
7.	Dr. G B Nair Executive Director Translational Health Science & Technology Institute Plot No. 496, Phase-III, Udyog Viharr Gurgaon-122016	Member

8.	Prof. T P Singh Distinguished Biotechnology Professor (DBT) Department of Biophysics All India Institute of Medical Sciences (AIIMS) Ansari Nagar New Delhi-110029	Member
9.	Prof. Joel Sussman Department of Structural Biology The Weizmann Institute of Science Rehovot, Israel 76100	Member
10.	Prof. Keiichi Namba Proteonic NanoMachine Group Graduate School of Frontier Biosciences Osaka University, 1-3 Yamadaoka, Suita, Osaka-565-0871 Japan	Member
11.	Prof. R Venkata Rao Vice Chancellor National Law School of India University Teachers Colony, Chandra Layout Bangalore-560242 Karnataka	Member
12.	Dr. Samudrala Gourinath School of Life Sciences Jawaharlal Nehru University New Delhi – 110 067	Special Invitee
13.	Dr. Vinay K Nandicoori National Institute of Immunology Aruna Asaf Ali Marg New Delhi – 110 067	Special Invitee
14.	Dr. Niranjan Chakraborty National Institute of Plant Genome Research Aruna Asaf Ali Marg New Delhi-110067	Special Invitee
15.	Dr. Dinakar M Salunke Executive Director Regional Centre for Biotechnology 180, Udyog Vihar, Phase-I Gurgaon-122016	Member Secretary

Executive Committee

1.	Dr. Dinakar M Salunke Executive Director Regional Centre for Biotechnology Gurgaon	Chairperson
2.	Mr. Shigeru Aoyagi Director & UNESCO representative to Bhutan, India, Maldives and Sri Lanka (UNESCO Office, New Delhi)	Member
3.	Mr. Amit Khare Joint Secretary Ministry of Human Resource Development Govt. of India, New Delhi	Member
4.	Mr. S Sinha Advisor, Dept. of Biotechnology Ministry of Science and Technology Govt. of India New Delhi	Member
5.	Mr. Tanmaya Lal Joint Secretary (UNES) Ministry of External Affairs New Delhi	Member
6.	Dr. Satyajit Rath Senior Scientist National Institute of Immunology Aruna Asaf Ali Marg, JNU Campus New Dellhi-110067	Special Invitee

Finance Sub-Committee

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

Scientific Personnel

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Associate Professor

Dr. Prasenjit Guchhait
Dr. Deepak T Nair

Assistant Professor

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Dr. Avinash Bajaj
Dr. Vengadesan Krishnan
Dr. Tushar Kanti Maiti
Dr. C V Srikanth
Dr. Sam Jacob Mathew
Dr. Saikat Bhattacharjee
Dr. Priyadarshini Chatterjee
Dr. Deepti Jain
Dr. Divya Chandran

International Adjunct Faculty

Dr. Falguni Sen

Young Investigators

Dr. Smriti Verma
Dr. Sushmita Bhattacharya
Dr. Masum Saini
Dr. Sheetal Chawla
Dr. Megha Kumar
Dr. Bornali Gohain
Dr. Amit Kumar Yadav
Dr. Ankur Kulshreshtha
Dr. Vaibhav K. Pandya

Wellcome DBT Early Career Fellows

Dr. Pushpa Kumari

Research Associates

Dr. Mukesh Kumar
Dr. Amit Kumar
Dr. Manish
Dr. Ankita Pandey
Dr. Sandhya Bansal
Dr. Jewel Jameeta Noor
Dr. Mritika Sen Gupta
Dr. Deepak Kumar Jangir

Research Fellows

Ms. Abha Jain
Mr. Sagar Mahale
Mr. Vedagopuram Sreekanth
Mr. Harsh Kumar
Ms. Harmeet Kaur
Ms. Pranita Hanpude
Mr. Pergu Rajaiah
Mr. Gowtham Kumar Annarapu
Mr. Somnath Kundu
Ms. Gayatree Mohapatra
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Mr. Avinash Gupta
Ms. Navneet Kaur
Ms. Varha Komala
Mr. Pavit Kumar
Ms. Neha Khare
Ms. Anuska Das

Management Personnel

Executive Director

Dr. Dinakar M Salunke

Academic

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Dr. B Chandrasekar

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Ms. Vaishali Mangla

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Mr. Biju Mathew

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Mr. Sanjeev Kumar Rana
Mr. Sudhir Kumar

Engineering and Technical

Executive Engineer

Mr. Ramesh Kumar Rathore

Technical Officer

Mr. Mahfooz Alam

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Mr. Suneel Prajapati

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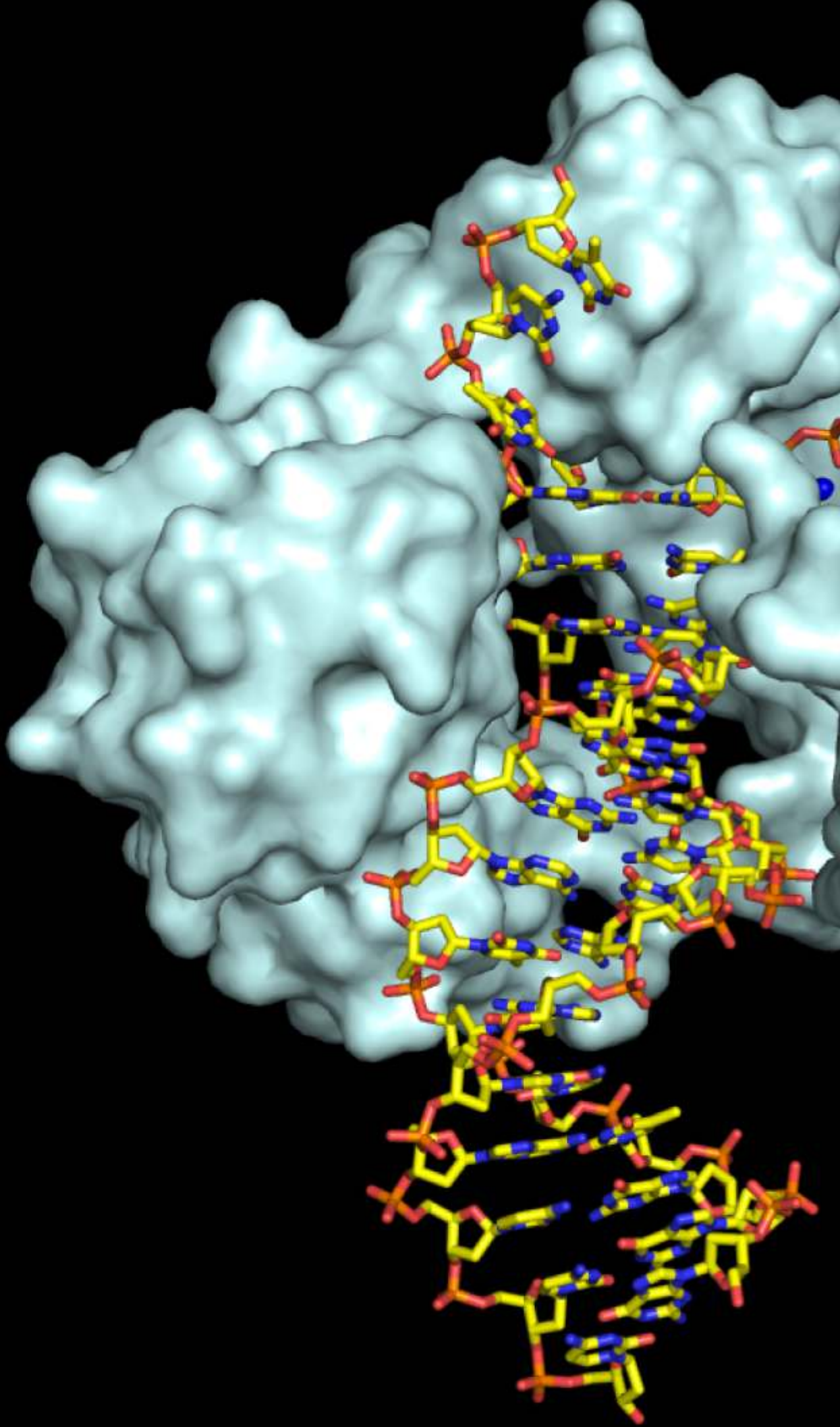
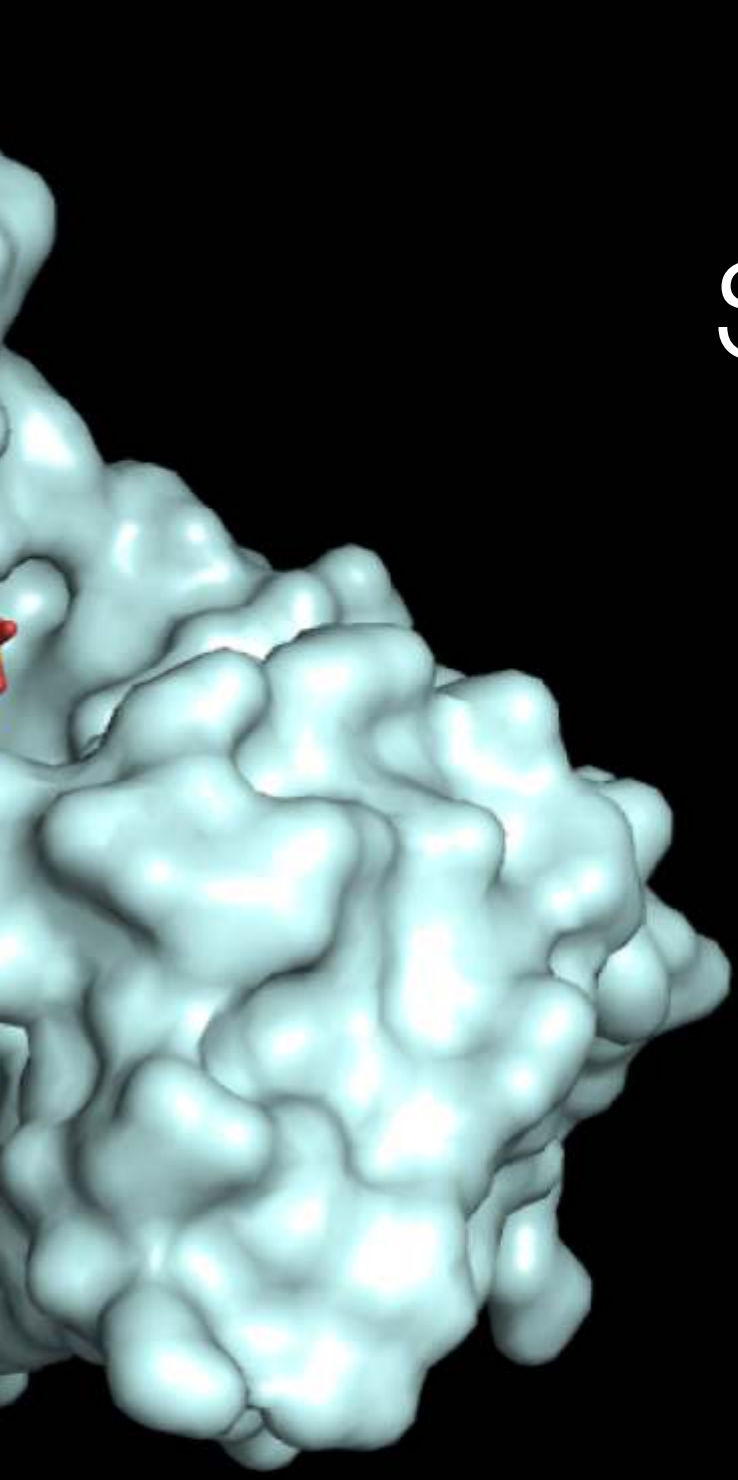
Junior Consultant

Mr. Ratindra Nath Chatterjee





Snapshots

Independence Day celebrations

Poster session during PAC meeting

Fresher's Welcome

Permanent Campus
Biotech Science Cluster
Faridabad

About RCB

The Regional Centre for Biotechnology (RCB) is a newly established institution of education, training & research in the National Capital Region (NCR) of Delhi by the Department of Biotechnology, Government of India under an agreement with UNESCO. RCB is designed to be a centre of excellence in biotechnology with intimate contributions from the countries of the region and academic institutions from the rest of the world. It provides a meeting place where innovation, enterprise, and industrial development will germinate. This Centre would be beneficial to all countries in the region including India in carrying out biotechnology research of highest caliber and developing knowledge-rich highly skilled human resource. Biotechnology being essentially global, the partnerships are as much within as across countries. Association with UNESCO essentially expands the opportunities to create world class education and research and provides seeds of global cooperation.

UNESCO
United Nations
Educational, Scientific and
Cultural Organization

REGIONAL CENTRE FOR BIOTECHNOLOGY
an institution of education, training and research

Established by the Dept. of Biotechnology, Govt. of India
Under the Auspices of UNESCO
180, Udyog Vihar, Phase 1, Gurgaon - 122016 India • Website: www.rcb.res.in