United Nations
Educational, Scientific and
Cultural Organization

REGIONAL CENTRE FOR BIOTECHNOLOGY

an institution of education, training and research

(Established by the Dept. of Biotechnology, Govt of India under the auspices of UNESCO)

Annual Report
2011-2012

Contents

Preamble

It is indeed a great pleasure to report substantial developments and growth at the Regional Centre for Biotechnology this year considering the limitations of functioning from an interim campus in Gurgaon. While the construction of the laboratory building in the Faridabad campus is in full swing, consolidation of academic and research programmes at the Gurgaon campus is underway. As efforts are being made to foray into new multidisciplinary facets of biotech science, the small beginning made in the field of biomedical sciences last year got accelerated.

Towards addressing the physico-chemical principles of allergy, the seed proteome of eggplant *(Solanum melongena)* was subjected to allergy screening. Structure of one of these proteins, EP1, was determined *ab initio* at 1.5 Å resolution. While continuing the work on degeneracy of antigen recognition, analyses of the conformational transitions of the germ line antibody receptor before antigen binding, upon binding and after release were carried out using molecular dynamics simulations in *silico* suggesting that the acquired rigidity after antigen removal may be the first step in affinity maturation in favour of that antigen. Under the theme of cell division, namely the transition from metaphase to anaphase, a process monitored by the spindle assembly checkpoint, efforts were made towards delineating the molecular mechanism of inactivation of the spindle assembly checkpoint driven by the Light Intermediate Chain 1 subunit of cytoplasmic dynein. In the research projects focusing on lipid-based drug delivery system for cancer, synthesis of a series of designed molecules was achieved and their *in vitro* anticancer activities were tested. The ongoing research project on bacterial surface proteins aimed at understanding pilus architecture, assembly, and pili-mediated host interaction has shown substantial preliminary leads. Towards addressing the molecular mechanisms in the ubiquitin- mediated signalling in cellular pathways, the preliminary structural investigations on a protein associated with BRCA1 and its interactions with ubiquitin have been carried out. Towards understanding the molecular mechanisms of infectious and idiopathic inflammation, the focus has been on the role of post-translational modifications, such as SUMOylation, in the infection and inflammation caused during *Salmonella* infection. Research programme on hemolysis and regulation of thrombosis in hemolytic conditions, the binding of extracellular hemoglobin (Hb) to the plasma protein von Willebrand factor (VWF) that makes VWF hyper-reactive to culminate in platelet adhesion and aggregation is being actively pursued.

Educational and training activities have been initiated in right earnest. Two workshops were organized, one on technology platforms for molecular crosstalks in modern biology and another on eukaryotic model organisms. These involved lectures and hands-on training by eminent experts. Goa University and the Manipal University have recognized RCB as their Research Centre for the purpose of research by fellows under the supervision of RCB faculty leading to Ph D degree. This will provide huge impetus for education and research at the Centre while we await Parliament to enact the RCB Bill, enabling the Centre to fully equip itself for diverse multi-disciplinary academic programmes as mandated.

A new member has joined RCB Faculty during this period to initiate studies on specific contractile proteins in cell culture and in model organisms. A good number of RCB Young Investigator (RCB YI) Awardees, Junior Research Fellows, Post Doctoral Fellows, Research Associates and Project Assistants have strengthened the research arms of the RCB Faculty and all of them are dedicated towards taking the Regional Centre to newer heights. Technical Assistants and the Administrative Staff are extending healthy support to the Research hands. It is indeed very satisfying to report RCB's maturing into a functional institution of biotechnology due to the efforts of all involved.

Dinakar M. Salunke
Executive Director

Mandate of the Centre

Mandate of the Centre is to provide a platform for biotechnology education, training and research at the interface of multiple disciplines. The programmes of the Centre will be designed to create opportunities for students to engage in multi-disciplinary research where they learn biotech science while integrating engineering, medicine and science, to provide solutions for human and animal health, agriculture and environmental technologies.

The mission of the Centre is to create opportunities for multi-disciplinary education, training and research in biotechnology. 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 will also be 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 Co–operation (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.

Scientific Reports

Structural Biology of Regulatory Events in Physiological Processes

Principal Investigator	Dinakar M Salunke, Ph D
Young Investigators	Jasmita Gill, Ph D Alka Dwevedi, Ph D
Junior Research Fellows	Abha Jain Anamika Singh Harmeet Kaur
Collaborator	Akhilesh K Tyagi, Ph D, NIPGR

Theme of Research

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 during 2011-2012

The structural proteomics studies of plant seed allergens were continued. The seed proteome of eggplant *(Solanum melongena)* was subjected to allergy screen towards carrying out crystallographic analyses of food allergens. The seeds were processed by delipidification and proteins separated based on ammonium sulphate fractionation. The abundantly present proteins were then subjected to N-terminal sequencing in order to identify the proteins through homology based computational searches. The screening of non-redundant database assisted in identification of necessary relationships of these proteins with allergy. Two of the segregated proteins from *S. melongena*, EP1 and EP2, which showed sequence relationships with known allergenic proteins were purified and subjected to crystallization attempts.

Crystals of EP1 could be obtained that diffracted at 1.5 Å resolution at synchrotron source (BM14, ESRF, Grenoble). The preliminary characterization showed that the crystals belonged to space group R32 with cell parameters a = 119.4 Å, c = 158.0 Å, respectively. As no crystal structures of closely related proteins were available, structure determination was pursued by *ab initio* phasing. Single wavelength anomalous diffraction (SAD) using sulphur provided the solution to the phase problem and the structure was refined at 1.5 Å with the initial free R factor of 0.343. Two data sets at 1.5 Å and 2.18 Å were collected from the same crystal, which was flash-cooled at 100K. Sulfur sites were determined by PHENIX using Autosol with data from 40 Å to 2.18 Å. In total, 8 sites could be identified from the Bijvoet pair differences. The initial phases and their figures of merit were input into RESOLVE and auto-build. RESOLVE automatically traced 225 residues and the remainder of the structure was manually built. Refinement of the structure was done in REFMAC from CCP4 suite.

The final refinement cycle gave an overall R factor of 0.197 (R free = 0.218) for all data up to 1.5 Å resolution.

The overall structure of EP1 consists of 393 residues of which residues 180-198 and 274-293 are structurally disordered and show broken density whereas the core is clearly resolved. The structure of EP1 contains one monomer in the asymmetric unit with 48% solvent content. However, the size exclusion chromatography profile of EP1 suggested that the protein could exist as a trimer. Further analysis of the region around the asymmetric unit revealed that it exists as homotrimer. The three monomers of the trimer are related by the crystallographic threefold axis. The monomer within the subunit is composed of two similar domains further subdivided into a core and a loop subdomain. Each domain consists of 2 elements, a compact eight-stranded beta barrel having the “swiss roll” topology and an extended flexible fragment containing several short alpha helices. It demonstrated an interesting pseudo-R32 symmetry. This could be explained only by accepting the close similarity between amino- and carboxy- terminal halves of the subunit related by pseudo 2-fold axis and therefore show internal structural symmetry reflecting genetic duplication.

Structural fold analysis tells us that although there was a little difference, basic structure was close to those of 7S Adzuki bean, canavalin, Arah1 and phaseolin. All these proteins have very low sequence identity suggesting multiple and varied sequence can yield similar three- dimensional structures and also explains evolutionary divergence. Identification and description of amino acids that are conserved between domains and among vicilin can define the specific residues required for structural integrity, for proper polypeptide folding, or for ensuring some functional property.

Another family of proteins that is being worked upon in the context of allergy is rice expansins. Expansins form a large multi-gene family found in rice, wheat and other cereal genomes that are involved in the expansion of cell walls as a tissue grows. EXPA are most abundant in dicots, while EXPB are abundant in maize and other graminaceous plants. The present work emphasizes structural studies on rice pollen allergenicity caused due to EXPB present abundantly in pollen. This will help in understanding mechanism of protein allergenicity, which would contribute in improvement of allergy prediction, diagnosis, and management of its severity in individuals. Expression vectors corresponding to the four proteins were provided by Dr AK Tyagi, NIPGR, New Delhi. Protein expression corresponding to these was optimized with respect to various parameters like temperature, IPTG concentration, incubation time etc. Protein purification was done using Ni-NTA super flow resin. Optimization with respect to equilibration, washing and elution has been successfully completed. The proteins, which are effectively different subtypes of EXPB, so obtained, are in unfolded state. Optimization of their refolding and further processes towards crystallization attempts is in progress.

Continuing the work on degeneracy of antigen recognition in the antibody response, molecular dynamics (MD) simulations were performed on the complexes of primary antibodies and the corresponding antigens in different states. Analysis of the conformational transitions of a receptor before ligand binding, upon ligand binding and after ligand release can advance our understanding of receptor function. Immune complexes involving diverse antigens and the corresponding receptors could provide suitable model systems for analyzing such conformational transitions. Structures of antibody-antigen complexes describe diverse facets of antigen recognition and thus provide wide range of structural datasets for addressing mechanistic models of antigen encounter by primary antibodies using in *silico* approaches. MD simulations provide a powerful tool for the exploration of the conformational energy landscape accessible to biomolecules and are becoming an essential technique in structural biology. The key objective of this work is to analyse the conformational repertoire of germline antibody-antigen complexes to describe antibody dynamics and understand the likely effects of antigen interaction during primary immune response.

Two comprehensive datasets consisting of three-dimensional structures of antigen-free and antigen-bound germline antibodies 36-65 and BBE6.12H3 have provided insights into antibody diversity [Sethi *et al, Immunity* (2006) 24:429; Khan and Salunke, *J Immunol* (2012) 188:1819). We have analyzed MD simulations of antigen-free and antigen-bound states of germline antibodies 36-65 and BBE6.12H3. The data show that germline antibodies 36-65 and BBE6.12H3 exhibit similar mobility during the course of the simulation despite their ability to adopt contrasting mechanisms for germline repertoire expansion as shown in crystallographic studies. Additionally, antigen-bound states of germline antibodies 36-65 and BBE6.12H3 exhibit rigidity in their CDR antigen-interacting residues in the presence as well as absence of the antigen. These results suggest that antigen-bound CDR conformation of a germline antibody is a likely structural template or a prior memory of the antibody for that particular antigen and this acquired rigidity may be the first step in affinity maturation in favour of that antigen, supporting the well-established concept of high-affinity and specific binding of an antigen during affinity-maturation. The plan is to analyse other available germline antibody three-dimensional datasets exhibiting different mechanisms for antigen- recognition using MD to shed insights into antibody dynamics. The ultimate goal is to interpret physiological relevance from these data.

Future plans

Structural proteomics of plant seed allergens will be carried out. A pollen allergen from *Oryza sativa* is being studied towards crystallographic analysis and structure-function correlation. Complete processing of the seed proteomes of *Coffea arabica, Solanum melongena, Mucuna pruriens, Jatropha curcas, Papaver somniferum* and *Carum copticum* are being analysed through integrated approaches.

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.

Publications

Original peer-reviewed articles

1. Khan T and Salunke DM (2012). Structural elucidation of the mechanistic basis of degeneracy in the primary humoral response. *J Immunol* 188: 1819-27.

2. Tomar D, Khan T, Singh RR, Mishra S, Gupta S, Surolia A, Salunke DM (2012). Crystallographic study of novel transthyretin ligands exhibiting negative-cooperativity between two thyroxine binding sites. *PLoS One* 7: e43522.

3. Bhowmick A and Salunke DM (2012). Limited conformational flexibility in the paratope may be responsible for degenerate specificity of HIV epitope recognition. *Int Immunol* (in press)

Mechanisms of Cell Division and Cellular Dynamics

Principal Investigator	Sivaram V S Mylavarapu, Ph D
Young Investigator	Sharmishtha Samantaray, Ph D
Junior Research Fellows	Sagar Mahalle Harsh Kumar Rajaiah Pergu

Theme of Research

Dynamic cellular events are achieved through a tightly regulated interplay of biomolecules, a precise understanding of which is essential to understand human health and combat disease. We will examine molecular mechanisms underlying the basic physiological processes of cell division and intercellular communication.

Objectives

Fidelity of mammalian cell division is ensured through tight molecular regulation, mis- regulation of or slippage through, which leads to aberrant mitosis, chromosome mis- segregation and aneuploidy, which are well-established precursors to major diseases like cancer and polycystic kidney disease. Elucidation of the molecular mechanisms of mitotic regulation is imperative to understand the basis for asymmetric stem cell division as well as for potential therapeutic intervention in major diseases.

We wish to uncover the molecular mechanism of Spindle Assembly Checkpoint (SAC) inactivation by the Light Intermediate Chain 1 (LIC1) subunit of cytoplasmic dynein. We will determine the mitotic LIC1 interactome, dissect mitotic LIC1 phosphoregulation and probe the structural basis for LIC1 function. We also wish to provide a molecular explanation for the distinct mitotic roles of LIC isoforms -LIC1 in metaphase exit and LIC2 in completion of cytokinesis. In addition, 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 during 2011-2012

The Spindle Assembly Checkpoint (SAC) ensures that sister chromatids of all chromosomes are equally segregated in anaphase to future daughter cells by arresting cells in metaphase until its conditions - bipolar spindle attachment of all sister chromatids and subsequent inter- kinetochore tension - are satisfied. SAC effector proteins are subsequently removed (stripped) from kinetochores by the pluripotent molecular motor dynein to achieve checkpoint inactivation and facilitate anaphase onset. We had demonstrated that the LIC1 subunit, as part of the molecular motor dynein is responsible for stripping of SAC effector proteins from kinetochores at metaphase in a phosphoregulated manner. We are now focussed on dissecting the molecular mechanism of SAC inactivation along the following lines of investigation: 1) determine the mitotic LIC1 interactome to identify potential collaborators of LIC1, 2) determine the structural basis for LIC1 function and 3) determine the significance of multi-site phosphorylation of LIC1.

We have generated a series of mammalian expression clones of rat LIC1 (rLIC1; myc-rLIC1 can functionally substitute for human LIC1 - hLIC1 - in SAC inactivation). These clones impart either biochemical affinity purification tags (myc, 3X FLAG-YFP, Tandem Affinity Purification – TAP) for use in proteomic identification of the mitotic LIC1 interactome, genetically encoded fluorescent

tags (mCherry, YFP, Dendra etc.) for live-cell imaging, or both. We are presently establishing stably expressing cell lines expressing all the above constructs individually. Transiently over-expressed wild-type mCherry-rLIC1 localized to vesicles (*Fig. 1a*) which showed vectorial motility in interphase. mCherry-rLIC1 could also rescue the metaphase arrest caused due to siRNA-mediated depletion of endogenous hLIC1 as efficiently as myc-rLIC1 (*Fig. 1b*). These results suggest that introduction of the mCherry tag does not detectably impede rLIC1 function.

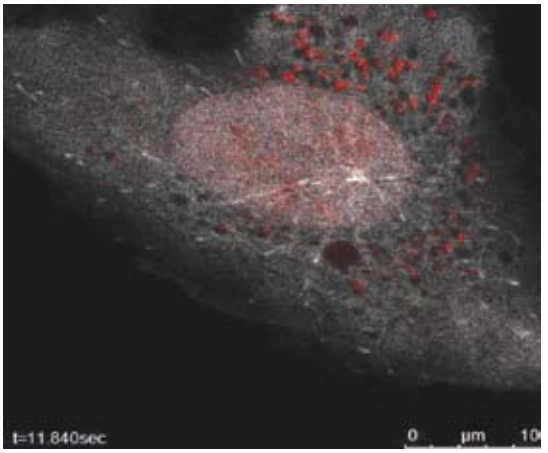


Fig. 1a. *Snapshot from a movie depicting EB1-GFP in white and mCherry-rLIC1 in red expressed in an RPE1 cell line. mCherry-rLIC1 is seen concentrated on foci corresponding to vesicles around the nucleus. Some mCherry-coated vesicles are seen moving linearly in the movie, suggesting vectorial movement of dynein along microtubules.*

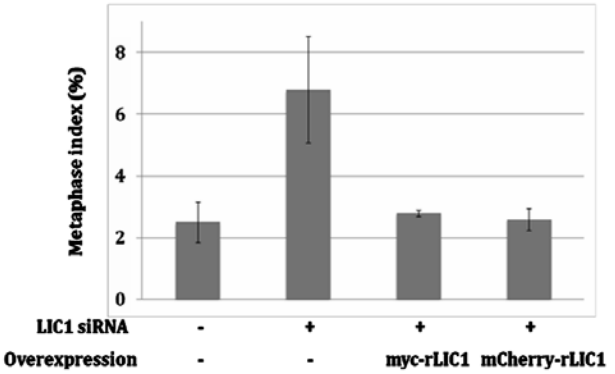


Fig. 1b. *mCherry-LIC1 can functionally rescue metaphase arrest of cells upon siRNA mediated depletion of endogenous LIC1, as efficiently as previously reported myc-rLIC1.*

In parallel, we are purifying recombinant rLIC1 protein for crystallization and structure determination by x-ray crystallography. Full length rLIC1 and several rationally truncated constructs were designed, based on consensus secondary structure predictions combined with primary protein sequence conservation (*Fig. 2a*). These constructs were cloned into a battery of recombinant expression plasmids (6-His-tagged, GST-tagged), expression conditions optimized and solubility of expressing constructs assessed. Of these constructs, the GST- tagged predicted helical “structural core” of rLIC1 (*Fig. 2a, rLIC1-SC*) expressed well, was soluble and about 80 – 90 % pure after proteolytic removal of the GST tag (*Fig. 2b*). Further purification by ion exchange and gel filtration chromatography is currently being optimized to obtain >95% pure protein for biophysical and structural analyses.

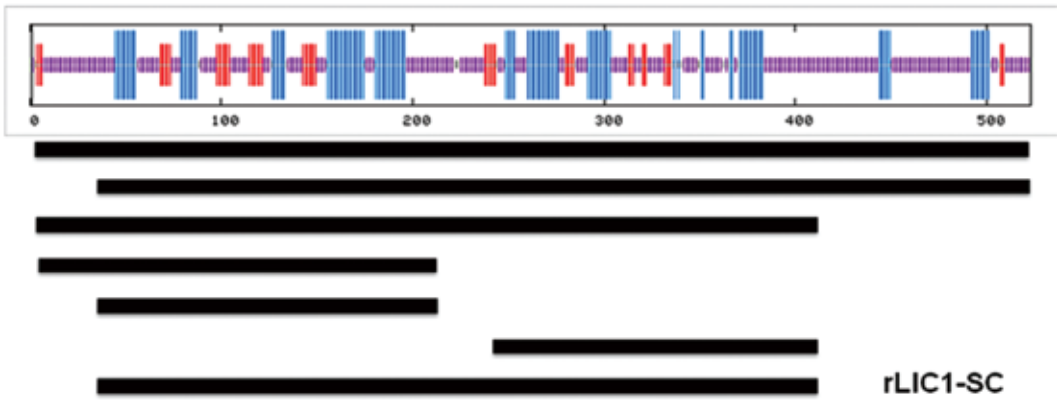


Fig 2a: *Top – consensus secondary structure prediction of rLIC1 (blue = helix, red = beta strand, purple = random coil). Bottom - Schematic of the various rLIC1 constructs designed and tested for expression and solubility. rLIC1-SC (structural core) was soluble and is being purified to homogeneity.*

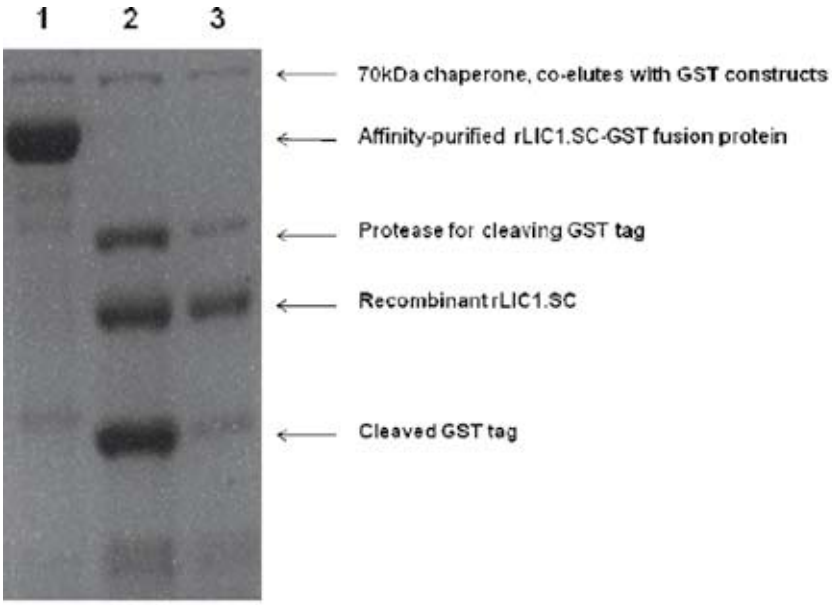


Fig. 2b: *Partial purification of recombinant rLIC1-SC-GST. Lane 1 – affinity-eluted fraction off a glutathione column; Lane 2 – sample after protease cleavage to cleave the GST tag off the protein; Lane 3 – cleaved protein passed over a glutathione column to subtract the cleaved GST (~80% pure protein).*

We are also exploring the differential regulation of mitotic dynein function by its LIC isoforms, LIC1 and LIC2. LIC1 and LIC2 have been implicated in distinct mitotic roles in mammalian cells -LIC1 for inactivation of the SAC to allow anaphase onset (as discussed above) and LIC2 in ensuring

completion of cytokinesis. The assembly of LIC1 and LIC2 into dynein is mutually exclusive - dynein contains a homodimer of either LIC1 or LIC2, but not both. Consistent with this, individual dynein complexes containing either LIC1 or LIC2 have been demonstrated. Despite their distinct and non-overlapping functions however, LIC1 and LIC2 share high sequence identity (~66%) and homology (~75%). Consensus secondary structure predictions (*Fig. 3a*) show a similar domain pattern, with two largely helical regions in the middle (predicted structural core) flanked by predicted unstructured regions towards the termini. Intriguingly, the structural cores also exhibit higher sequence similarity (~75%) as compared to the full-length proteins (~66%).

We therefore postulate that the central regions of both LIC1 and LIC2 constitute their relatively invariant structural scaffolds, while the other regions of higher sequence mismatch are responsible for unique biochemical interactions that drive their distinct functions. These differences in function may arise from changes in conformation, surface chemistries, or both. We are testing this hypothesis by attempting to functionally convert LIC1 into LIC2, by introducing regions of mutual sequence mismatch from LIC2 into LIC1. These protein chimeras will help identify regions specific to both LICs that are important for their respective functions in mitosis.

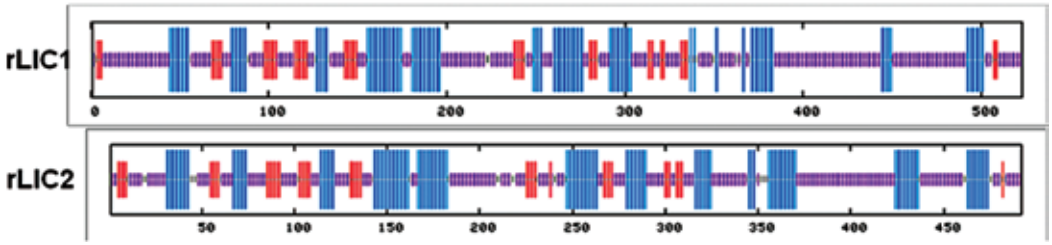
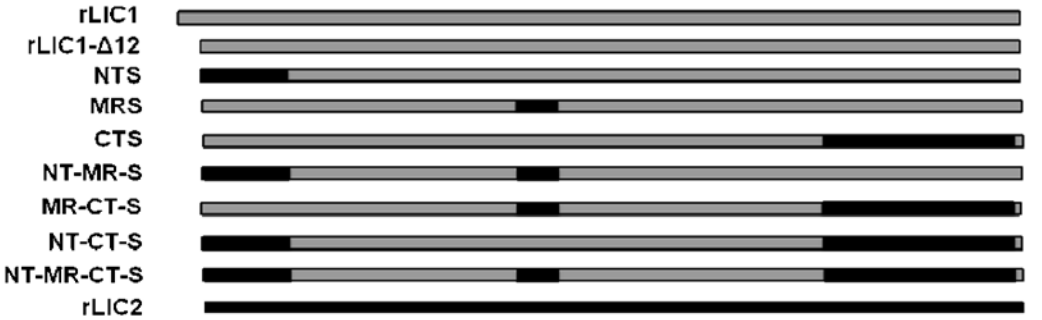


Fig. 3a: *Schematic of consensus secondary structure prediction for rLIC1 and rLIC2. Colour-coding as in fig. 2a.*

Accordingly, we have designed seven recombinant, hybrid rLIC constructs containing rationally selected regions of rLIC2 introduced into the rLIC1 template (*fig. 3b*). These hybrids encompass three regions of highest sequence dissimilarity observed between rLIC1 and rLIC2 – the N terminus, a small central region and the C-terminus – singly and in all possible combinations. Care has been taken not to disrupt predicted secondary structural elements. The chimeras have been cloned into both mammalian (under a pCMV promoter) and bacterial (GST-tagged) expression vectors. We have initiated functional studies on these chimeras to assay for loss of LIC1 function and/ or concomitant gain of LIC2 function.



Figu 3b: *Schematic of the various rLIC1-rLIC2 hybrid constructs generated.*

Future plans

Mammalian cell lines stably expressing tagged rLIC1 at near-endogenous levels will be used in both biochemical/ proteomic and imaging studies, which will be compared for mutual validation. Mitotic interaction partners of LIC1 will be identified from lysates of mitotically synchronized cells, obtained using cryogenic methods to preserve endogenous protein interactions. Immunoprecipitates from these lysates utilizing the affinity tag(s) on rLIC1 will be subjected to mass spectrometric analyses to identify the interactome of LIC1. Comparison with the interactome of a functionally incompetent phospho-mutant (S207A) will help identify the proteins that differentially bind to these LIC1 isoforms, which are also likely to be important for LIC1's ability to strip SAC proteins from kinetochores. These proteins would be individually knocked down in functional studies to assay for phenotypes similar to LIC1 depletion. In parallel, their spatio-temporal localization with LIC1 at kinetochores and/ or spindle poles in mitosis will be studied by high-resolution immunofluorescence and live- cell imaging. These studies would together give deep functional insights into the molecular mechanisms for dynein-mediated SAC inactivation.

For structural analyses, purified recombinant rLIC1 (structural core and other constructs) will be characterized by several biophysical assays. Retention time on gel filtration will give clues to the oligomeric status of the protein, which will be confirmed by dynamic light scattering and/ or analytical ultracentrifugation, if necessary. This is important because cytoplasmic dynein is believed to contain two copies of LIC. Limited proteolysis of purified rLIC1 will be used to identify compactly folded structural domains of rLIC1 that are resistant to proteolysis.

Such domains would be recombinantly expressed and purified, with the eventual aim of crystallization and three-dimensional structure determination. Determination of the atomic structure of LIC1 will enable rational mutagenesis of conserved *surface* residues of LIC1, which are strong candidates for mediating LIC1-partner protein interactions. The surface mutants will be assayed both for biochemical loss of binding partner(s) identified above and also for concomitant defects in SAC inactivation. Together, the biochemical/ proteomic, structural and functional studies outlined above will serve to illuminate the detailed molecular mechanism of LIC1-mediated SAC silencing by dynein.

For studying the differential regulation of cytoplasmic dynein function by LIC1 and LIC2, the rLIC hybrids generated will be expressed in human cell lines following siRNA-mediated depletion of endogenous hLIC1, which leads to arrest in metaphase. Those hybrids that fail to mimic functional rescue of the metaphase arrest (similar to rLIC1) would be categorized as loss-of-LIC1-function hybrids. These hybrids would subsequently be assayed for gain-of- LIC2 function, by testing for their ability to rescue the (previously reported) arrest of cells in cytokinesis upon siRNA-mediated LIC2 depletion.

The hybrids that lose LIC1 function and simultaneously gain LIC2 function would be further analyzed. Biochemical/ proteomic assays from mammalian cell lysates expressing these hybrids (as described above) would be used to ascertain the change in the interactome of the hybrid in comparison to the LIC1 interactome. We will similarly determine the LIC2 interactome from cytokinesis-enriched lysates. Upon comparison, we expect to see loss of LIC1 interactor(s) and gain of LIC2 interactor(s) when expressing the hybrids. Expression and affinity purification of the interesting hybrids using the GST tag attached will enable biophysical and structural characterization. The structural model obtained for LIC1 above would also aid in building a structural model for LIC2 by homology modelling, given the high degree of sequence identity (66%) between LIC1 and LIC2. It is also possible that the large scale changes made in these hybrids might render some of them non-functional due to misfolding or loss of conformation. In this eventuality, we will fine-tune our mutagenesis approach and mutate only select conserved residues from the swap regions in LIC1 (as opposed to swapping the entire

region) and assay for function as detailed above. In conjunction, the data generated here would help ascertain the molecular determinants that govern the divergent mitotic functions of cytoplasmic dynein containing LIC1 or LIC2.

In the long term, we aim to elucidate key molecular mechanisms that are important for regulating metaphase exit as well as for completion of cytokinesis using the multidisciplinary approach outlined above. We will also investigate the biogenesis and function of novel modes of intercellular communication in mammalian cells.

Engineering of Nanomaterials for Biomedical Applications

Principal Investigator	Avinash Bajaj, Ph D
Project Associates	Ashima Singh, Ph D Manish Singh, Ph D Sandhya Bansal, Ph D
Junior Research Fellows	Vedagopuram Sreekanth Somnath Kundu
Project Assistant	Priyanshu Bhargava

Theme of Research

We are using an interdisciplinary approach involving synthetic chemistry, cell biology and nanotechnology to address challenges in the area of cancer biology and develop nanomaterials for drug delivery, gene therapy and combination therapy.

Objectives

- Engineering of nanomaterials for drug delivery and gene therapy
- Exploiting nanomaterials for cancer combination therapy

Progress during 2011-2012

We are studying the use of lipidated nanomaterials for drug delivery. Bile acids are endogenous steroidal molecules which undergo entero-hepatic circulation. Conjugation of anticancer drugs to bile acids is anticipated to increase bioavailability of poorly bio-available drugs and improve tumor targeting. The bile acid molecules will also help in liposomal formulation that can help liposomal drug formulations to avoid entero-hepatic circulation and to enter into lymphatic circulation on oral absorption.

We have synthesized two series of biocompatible carrier based Bile Acid-Tamoxifen conjugates having acid and amine head groups: Lithocholic Acid-Tamoxifen **(LA-Tam$_1$- Ad&LA-Tam$_1$-Am)**, Deoxycholic Acid-Tamoxifen **(DCA-Tam$_2$-Ad&DCA-Tam$_2$-Am)**, Cholic Acid-Tamoxifen **(CA-Tam$_3$-Ad&CA-Tam$_3$-Am)** possessing 1, 2, 3 tamoxifen drug molecules respectively. The anticancer activities of these compounds were performed in breast cancer cell lines 4T1, MCF-7, T47D, MDA-MB-231 using MTT assay. Cholic Acid- Tamoxifen conjugate **(CA-Tam$_3$-Am)** with amine head group having three tamoxifen molecules attached to cholic acid is the most effective, causing 50% and 90% anticancer activity at 5 μM and 10 μM respectively, whereas tamoxifen (Tam) alone showed only 40% anticancer activity at 10 μM. Annexin V-FITC-PI apoptotic assay showed two-fold increase in apoptotic 4T1 cells by **CA-Tam$_3$-Am** as compared to tamoxifen alone. 50% of early apoptotic cells were observed upon treatment with **CA-Tam$_3$-Am** (10 μM equivalent of tamoxifen concentration) whereas treatment of 10 μM tamoxifen alone showed only 23% of early apoptotic cells.

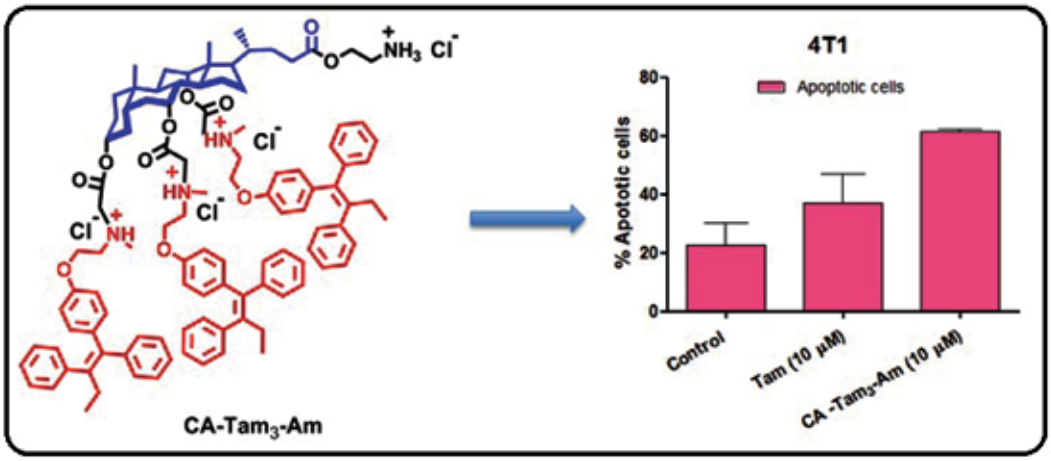


Fig 1. *Molecular structure of Cholic Acid Tamoxifen conjugate* ***(CA-Tam$_3$-Am)*** *having amine head group and Annexin-FITC based apoptotic assay using* ***TamNHMe*** *and* ***CA-Tam$_3$-Am*** *in 4T1 cells.*

To explore the interactions of tamoxifen and bile acid tamoxifen conjugates, we studied the interactions of desmethylated tamoxifen **(Tam-NHMe)** and Cholic acid-Tamoxifen conjugate **CA-Tam$_3$-Am** with DPPC membranes, using DPH based fluorescence anisotropy ad Prodan based hydration studies. The incorporation of TamNHMe in DPPC membranes causes a little increase in anisotropy of DPPC membranes in gel phase, which does not change further upon incorporation of increasing amount of desmethylated tamoxifen. **CA-Tam$_3$-Am** upon getting incorporated up to 20% in DPPC membranes does not make drastic change in membrane rigidity, but upon further increase in % mol incorporation there is dramatic decrease in membrane rigidity of DPPC membranes. Initial increase in anisotropy values indicates more rigidity of membranes that may be due to strong hydrogen bonding between amine head group of **CA-Tam$_3$-Am** and phospholipids. Further increases in incorporation of **CA-Tam$_3$- Am** fluidize DPPC membranes and making DPPC membranes dis-ordered in their gel phase.

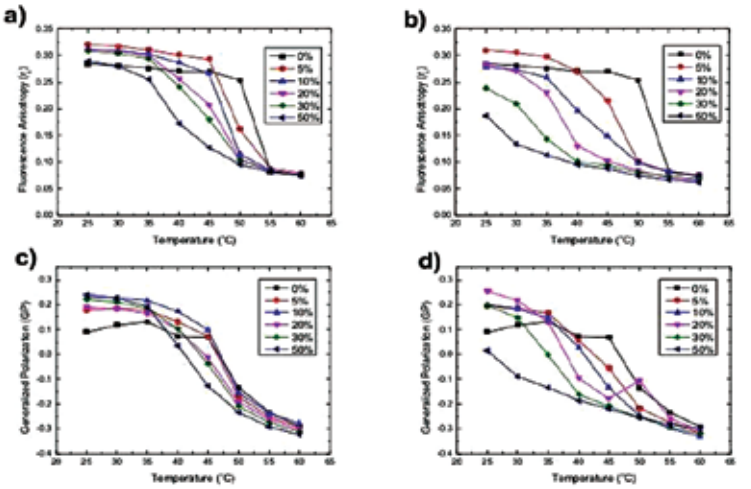


Fig 2. *Effect of temperature on membrane rigidity (a, b) and hydration (c, d) of DPPC membranes*

*upon incorporation of **Tam-NHMe** (a, c), and **CA-Tam$_3$-Am** conjugate (b, d).*

There is no change in the anisotropy values in liquid crystalline phase upon incorporation of **TamNHMe** and **CA-Tam$_3$-Am**, suggesting same fluidity of liposomes above phase transition temperature. Tam and **CA-Tam$_3$-Am** blend well in DPPC membranes and DPH experiences similar kind of environment.

Incorporation of **TamNHMe** inside the DPPC liposomes increases generalized polarization of Prodan. Increase in polarization indicates strong association between the hydrophobic tails of DPPC and **TamNHMe** making membrane surface less hydrophilic; and similar trend was observed above phase transition temperature. The strong interactions between **TamNHMe** and hydrophobic tails also lead to a little increase in phase transition temperature as evident from Prodan studies. With increase in incorporation of **CA-Tam$_3$-Am**, there is increase in generalized polarization of Prodan up to 20%, where as further increase in incorporation of **CA-Tam$_3$-Am**, decreases the generalized polarization. This initial increase followed by decrease in membrane hydration upon incorporation of **CA-Tam$_3$-Am** indicates that upon initial incorporation strong hydrogen-bonding between amine functionality of **CA-Tam$_3$-Am** and phospholipids dehydrate lipid surface as observed during anisotropy studies as well, whereas further increase in incorporation fluidize membranes in gel phase due to higher hydrophobicity of **CA-Tam$_3$-Am**.

We have also generated lipidated nanomaterials for gene therapy. We have synthesized a library of different cationic lipids based on lithocholic acid, deoxycholic acid, and cholic acid having 1, 2, 3 hydroxy groups on concave surface of their hydrophobic part. Cationic functional groups have been attached to head as well as tail regions of the bile acids. We are exploring use of biodegradable linkages like ester linkage or hydrazine linkage between cationic head group and hydrophobic parts. Hydrazone linkage between head group and hydrophobic part would help liposomes for early endosomal release of DNA/shRNA from complexes. In another library of cationic lipids, we are attaching cationic head group to tail region of bile acids. We would explore shRNA delivery efficacies of liposomal formulations in different cancer cell lines. First, to check DNA delivery efficacy, we would study transfection efficacies using EGFP-c3 plasmid DNA and β-galactosidase DNA. Cells will be incubated with lipoplexes containing these plasmid DNA; and transfection efficacies will be studied after different time intervals. We would then explore delivery of shRNA plasmids using these liposomal formulations. Different shRNAs against oncogenes like AKT, Polo kinase-1, CDK-1, Bcl-2 etc will be used for these studies. Cells will be incubated with different lipid-shRNA complexes, and effect of knock down of genes using shRNA will be explored after 24h, 48h, 72h and 96h. The effect of knock down will be studied by RT-PCR and Western blotting studies. The suppression of different oncogenes is known to show anticancer activities. Therefore, we would explore anticancer properties of lipid-shRNA complexes in different cancer cell lines.

Future plans

Combination therapy is important for better long-term prognosis and to decrease side effects due to the molecular complexity of many diseases. Combination therapy usually involves the simultaneous administration of two or more pharmacologically active agents or combination of different types of therapy (like chemotherapy and radiotherapy). Several diseases like malaria, HIV/AIDS and cancer have been routinely treated with combination therapy. Although the use of combination therapy for cancer treatment is well established, chemotherapeutic drugs are normally associated with challenges of severe side effects, less efficient targeting to cancer cells, and drug resistance of chemotherapeutic drugs. Therefore administration of a combination of drugs in liposomes, or a combination of drugs and genes would help in targeting multiple signaling pathways for effective therapy, and in reducing severe side effects of drugs. We hypothesize that development of bile

acid conjugates comprising drug delivery and DNA/shRNA delivery vehicles would transform cancer therapy by increasing efficacy of cutting down multiple pathways. We would like to engineer bile acid-drug-DNA complexes for effective combination therapy against cancer. Nanoparticles would be formulated from these bile acid conjugates, and would be studied for anticancer activities. After these initial studies, these complexes comprising drug-bile acid conjugates would be explored for combination therapy using therapeutic shRNA and DNA. Later, bile acid based complexes would be designed to deliver other cytotoxic drugs and genes to specific wild type and knockout cells to exploit the nucleo-cytoplasmic commutations and effect of oncogenes and tumor suppressor genes on anticancer therapeutics.

Publications

Original Peer-reviewed articles

1. §Rana S, Singla AK, Bajaj A, Elci SG, Miranda O, Mout R, Yan B, Jirik FR and Rotello VM (2012). Array-Based Sensing of Metastases and Metastatic Cells and Tissues Using Nanoparticle-Fluorescent Protein Conjugates *ACS Nano* 6:8233-40

2. §Biswas J, Bajaj A and Bhattacharya S (2011). Membranes of cationic gemini lipids based on cholesterol with hydroxyl headgroups and their interactions with DNA and phospholipid. *J Phys Chem* B 115: 478-86.

§ Workdone elsewhere, published during the reporting year.

Structural Biology of Bacterial Surface Proteins

Principal Investigator Vengadesan Krishnan, PhD

Junior Research Fellow Deepak Singh

Theme of Research

Many bacteria assemble a multitude of proteins and protein assemblies (e.g. pili) on their cell surface for adhesion to and colonization of host tissues, escape from innate immune responses and establish bacterial infections. The theme of research is to understand the adhesion and assembly mechanism of these surface proteins and assemblies.

Objectives

Understanding of the infectious process, especially at the molecular level, is crucial for the prevention and treatment of bacterial infections. Bacterial surface proteins and proteinaceous pilus fiber play vital roles in attachment, which is a critical first step in pathogenesis. The pilus assembly in Gram-negative bacteria has been well studied and shown to be chaperone mediated by non-covalent linking of protein subunits called pilins. Pili is being studied in Gram-positive bacteria for a decade and is shown to be sortase mediated by covalent linking of two or more pilins as demonstrated in *Corynebacterium diphtheria*. We are trying to understand pilus assembly and its adhesion mechanism in pathogenic and non-pathogenic bacteria using structural biology tools. Our long-term objectives are:

- To study structural aspects of bacterial surface proteinaceous assemblies and their host interactions.
- To understand structural and functional differences between pathogenic and non- pathogenic bacteria in surface proteins assembly and adherence mechanisms.

Progress during 2011-12

According to *Corynebacterium diphtheria* model, a major pilin forms the pilus shaft, while one minor pilin is at the base and also decorated along the pili and the other minor pilin is at the tip. The major pilins contain a conserved Lys residue near N-terminal in addition to C- terminal sorting signal consisting of LPXTG motif, which is present in all pilins. A pilus- specific sortase cleaves the pilin at a sorting motif between Thr and Pro and covalently links the C-terminal end to another pilin via a conserved Lys side chain. Finally, the cross-linked pilus fibers are covalently attached to the peptidoglycan cross bridge of the bacterial cell wall by a house keeping sortase. The story of minor pilins' incorporation on to the pilus fibers is still evolving. Interestingly, these pili have been discovered recently in beneficial bacteria such as probiotics, *Lactobacillus rhamnosus* GG (LGG) while traditionally attention is being paid to pathogenic bacterial pili. These probiotic pili have now emerged as possible facilitators of adhesion for probiotic colonization in the gut. However, detailed structural information is not yet available to enable an understanding of their role in colonization in the gut and beneficial effects.

In order to understand pili-mediated adhesion, pathogenesis and beneficial effects, we have initiated structural work on one of the most commonly used probiotics, LGG. LGG assembles two types of pili encoded by *spaCBA* and *spaFED* gene clusters on their surface. In *spaCBA* pilus, the *SpaA* appears to be the major pilin building up the pilus shaft, while *SpaB* and *SpaC* are minor pilins. Similarly, major pilin *SpaD* forms pilus shaft in *spaFED* pilus, with minor pilins *SpaE* and *SpaF*. Initial attempts for expression and purification have been done for few of these pilus proteins in small scale. The major

pilin, *SpaA* was purified in large scale using affinity and gel-filtration chromatographic techniques. Attempts to crystallize *SpaA* by screening various conditions using in-house nano-litre liquid handling robotic system (Mosquito) as well as manual crystallization set up yielded crystals in few conditions. Optimization of one of these conditions yielded crystals (shown in *Fig. 1*), which are suitable for diffraction. Since the *SpaA* has very poor sequence homology with known structures, attempts to solve its structure by *de novo* methods are in progress.

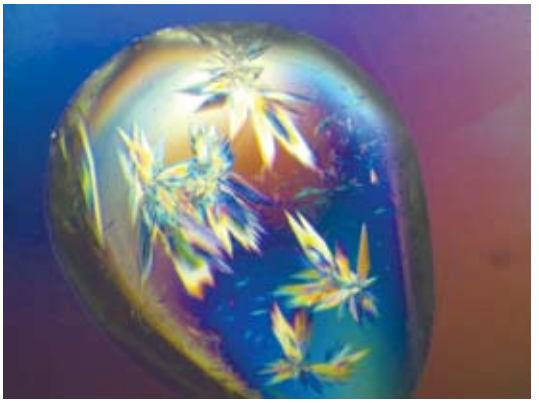


Fig. 1. *SpaA crystals*

Future plans

Adhesive pilins and pili-related proteins are potential candidates in protein-based vaccine attempts, and understanding their interactions in the assembly and with the host will provide crucial knowledge, which will facilitate vaccine design. We will continue and extend our structural and functional investigations to other pilins and sortases of LGG, and other pathogenic bacterial pili.

Publications

Original Peer-reviewed articles

1. §Mishra A, Devarajan B, Reardon ME, Dwivedi P, Krishnan V, Cisar JO, Das A, Narayana SV and Ton-That H (2011). Two autonomous structural modules in the fimbrial shaft adhesinFimA mediate *Actinomyces* interactions with streptococci and host cells during oral biofilm development. *Mol Microbiol* 81:1205-20.

2. §Khare B, Krishnan V, Rajashankar KR, I-Hsiu H, Xin M, Ton-That H and Narayana SV (2011). Structural differences between the *Streptococcus agalactiae* housekeeping and pilus-specific sortases: SrtA and SrtC1. *PLoS One* 6:e22995

§ Workdone elsewhere, published during the reporting year.

Investigating Molecular Mechanism in Ubiquitin Mediated Signalling in Cellular Pathways

Principal Investigator Tushar Kanti Maiti, Ph D

Young Investigator Sushmita Bhattacharya, Ph D

Junior Research Fellow Pranita Hanpude

Theme of Research

Ubiquitination is one of the most important post translational modifications involved in protein quality control in eukaryotes. The main theme of our research is to understand how ubiquitin is involved in different cellular pathways. The ubiquitin mediated signalling cascade is initiated by the formation of isopeptide linkage between target proteins and lysine residue of ubiquitin. In the ubiquitin signalling cascade there are two major events: ubiquitination, which leads to the conjugation of ubiquitin and deubiquitination, which leads to the deconjugation of ubiquitin. We are currently investigating the importance of the deubiquitination event in cellular function.

Objectives

Human genome analysis and mass spectrometric proteomic data reveal almost one hundred deubiquitinating enzymes. We would like to investigate the molecular basis of substrate recognition of deubiquitinating enzymes and their involvement in cellular functions like protein degradation, histone modification and endocytosis of plasma membrane proteins. The main objective of our investigation is to study the mechanistic detail of how the deubiquitinating enzymes recognize their substrate, and how these enzymes discriminate between different types of ubiquitin chains. It has been revealed that the dysregulation of deubiquitinating enzymes leads to diseases like Parkinsons, Alzheimers, Ataxia, heart disease and different types of cancer. Our aims are to understand the possible molecular mechanisms underlying these diseases.

Progress during 2011-12

BRCA1-associated protein-1 (BAP1), a member of Ubiquitin-C-terminal Hydrolases family of deubiquitinating enzymes, was discovered through its interaction with the RING finger domain of tumor suppressor protein BRCA1. BRCA1 forms a heterodimer with BARD1 protein and shows ubiquitin ligase (E3) activity. BAP1 interferes with BRCA1 ligase function. It has been postulated that BAP1 deubiquitinates auto-ubiquitinated BRCA1, but there is no clear evidence for this hypothesis. Unlike other UCH's, this protein contains UCH domain and nuclear localization signal domain. It has been demonstrated that both deubiquitination and nuclear localization are required for BAP1 mediated tumor suppression. BAP1 also interacts with transcription regulator Host Cell factor 1 (HCF1) without affecting its DUB activity. The association of BAP1 with HCF1 and regulation of other transcription factors implies that it has a role in chromatin modifications. On the other hand, BAP1 synergistically works with tumor suppressor gene BRCA1 and enhances its tumor suppression activity. Mutation of BAP1 leads to several forms of cancer like breast cancer and lung cancer. Recent studies show that germline mutations of BAP1 gene predispose to melanocytic tumors, mesothelioma and uveal melanomas. Considering all these properties of BAP1, it is pertinent to say it has diverse physiological roles in controlling cellular function.

Here we aim to explore the mechanism involved in BAP1 function keeping in mind some fundamental questions: Why is deubiquitinating activity important for tumor suppression? How do the mutations alter its function?

Towards addressing these questions, we have undertaken cloning, expression and purification studies of BAP1 protein. The full length and UCH domain of the BAP1 protein (1-240) were sub-cloned into pET 28a and pGEX-6PI vector according to the standard cloning protocol. The UCH domain (1-240) protein was over-expressed and purified using slandered GST purification protocol. The enzymatic activity of the protein was performed by Ub-AMC assay. This purified protein will be used for biophysical and structural experiments. C91S, A95D and G178V mutants were generated following the site directed Quick-change Mutagenesis protocol using pGEX-6PI–BAP1 (240) as a template. The proteins were purified according to GST-purification protocol as mentioned above. The purified protein will be used for functional analysis.

We have also initiated purification of Ub-VMe for making a suicidal complex of BAP1 and Ub-VMe. We are aiming to study the ubiquitin recognition of BAP1 protein. We have designed to make a covalent suicidal complex of BAP1 using the following protocol. The BAP1-UbVMe will be purified and subsequently used for structural studies. The methods involve several steps that include 1) preparation of Ub-MESNa, 2) synthesis of Gly-VMe and 3) synthesis and purification of Ub-VMe.

Ub-MESNa was obtained according to the scheme presented in the Figure 1. Ubiquitin was cloned into pTYB1 vector and expressed in *E*. coli Rosetta cells. The cells were grown in ampicillin and chloramphenicol antibiotic condition and induction was performed in 1mM IPTG at 18^0C for 18h. The cells were harvested and homogenized using the Panda homogenizer. The supernatant after centrifugation was loaded on to a chitin-column, which had been previously equilibrated with 50mM MES-acetate buffer. The column was washed with the same buffer having 40mM MESNa. All the purification steps were done at 4^0C. The column was kept at 37^0C for 16-18h and the Ub-MESNa was eluted with 50mM MES-acetate buffer containing 40mM MESNa. This UbMESNa was subsequently used for Ub-VMe formation without further purification.

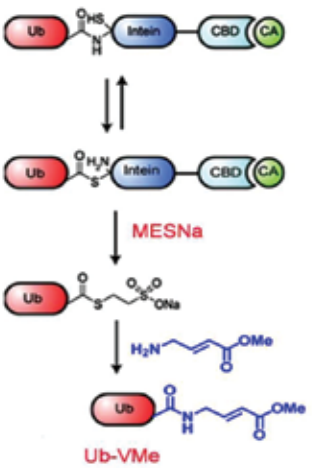


Fig.1: *Scheme for the synthesis of Ub-VMe*

Gly-vinyl methyl ester was synthesized according to the scheme presented in Figure 2.

Fig. 2: *Scheme for the synthesis of Gly-VMe*

Ub-VMe was purified according to the method described previously in the literature. In brief, molar excess of tosyl salt of Gly-VMe was mixed with Ub-MESNa at pH 8.0 in the presence of N-hydroxyl succinamide. The reaction mixture was kept at room temperature for 10-12h and then dialyzed against 50mM acetate buffer of pH 4.5. The Ub-VMe was purified using a Mono S-cation exchange column. The reactivity of Mono-S column eluted reactions was tested with BAP1 enzyme.

We further examined the importance of Alanine in the position at 95 and 178 with respect to its catalytic function. A95D and G178V mutants of BAP1 show a loss of tumor suppression activity. One interesting point is that in both cases the catalytic activity of BAP1 is reduced several folds. The reason behind the loss of catalytic activity is not clearly understood. The importance of alanine and glycine in position 95 and 178 respectively will be studied. We will replace the alanine at 95 or glycine at 178 position with different types of amino acids like basic (Lys, Arg, His), acidic (Asp and Glu), hydrophobic (Phe, Ile and Leu) and polar (Ser, Cys, Asn and Gln) amino acids. The consequence of the insertion of these amino acids may change the catalytic activity due to blocking the active site or perhaps due to disruption of secondary structure. How these mutations affect its protein interacting map will be studied by proteomic analyses. So far we have made Asp, Gly and Phe mutants in the Ala95 position. The mutants will be overexpressed and purified and subsequently we will measure their enzymatic activity, stability and effect on substrate recognition.

Future plans

Our future goal in this direction is to understand how somatic and germline mutations of BAP1 gene affect the ubiquitin pathway. We will also be targeting mechanistic and functional studies of deubiquitinating enzymes involved in the DNA repair mechanism.

Publications

Original Peer-reviewed articles

1. [§]Maiti TK, Yamada K, Inoue K, Kandori H (2012) L105K mutant of proteorhodopsin. *Biochemistry* 51: 3198-04.

2. [§]Boudreaux D, Chaney J, Maiti TK, Das C (2012) Contribution of active site glutamine to rate enhancement in ubiquitin C-terminal hydrolases *FEBS J* 279:1106-18.

3. [§]Maiti TK, Permaul M, Mahanic C, Mauney S, Das C (2011) Crystal Structure of Catalytic Domain of UCHL5, a Proteasome Associated Deubiquitinase, Reveals an Auto-Inhibited Conformation of the Enzyme. *FEBS J* 278: 4917-26.

[§] Workdone elsewhere, published during the reporting year.

Studies on Biology of Infectious and Idiopathic Inflammation of the Gut

Principal Investigator Chittur V Srikanth, Ph D

Young Investigator Smriti Verma, Ph D

Junior Research Fellow Gayatree Mohapatra

Theme of Research

The focus of our lab is to understand the molecular mechanism of infection and inflammation caused by the gastric pathogen *Salmonella enterica* serovar typhimurium, one of the most frequent causes of acute gastroenteritis. Moreover salmonellosis simulates ulcerative colitis clinically, histopathologically and radiologically, thus making it a very good model organism to understand these diseases as well.

Objectives

Salmonella is one of the most frequent causes of acute gastroenteritis in humans. The disease manifestation results in massive neutrophil infiltration at the site of infection. Remarkably this is a phenotype also seen in several forms of autoimmune disorders of the gut. 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. We would like to probe the pathways of the gastrointestinal inflammatory conditions to unearth other unidentified mechanisms that are akin in these states of inflammation. In these studies our main objectives will be to: (1) identify novel bacterial virulence proteins that mediate inflammatory pathways, (2) examine pathways that get affected during infections (including post translational modifications such as SUMOylation alteration), (3) test if the identified pathways are also operational during states of auto-immune disorders. Thus, this study may lead to the discovery of novel biochemical pathways that will advance the development of therapeutic approaches to treatment of intestinal inflammation.

Progress during 2011-2012

Preliminary experiments carried out to understand the global SUMOylation profiles of *Salmonella* infected epithelial cells compared to untreated cells revealed an interesting phenomenon involving the ubiquitin like post-translational modifier, SUMO. Humans have 3 different forms of SUMO (SUMO1, SUMO2 and SUMO3) that could be linked to hundreds of different target proteins undergoing post translational modification. The conjugation occurs at lysine residue of the consensus motif (ψKxD/E, where ψ is a hydrophobic residue, K the target lysine X any amino acid and D/E any acidic residue).

In our experiments which we call as global SUMOylation profile assays, we infected human colonic cell line, HCT8 with wildtype *Salmonella* strain (SL1344), lysed the cells in the presence of various protease inhibitors and deSUMOlase inhibitors at 3 hours (post infection) and subjected the samples for immunoblotting with anti SUMO1 and SUMO2/3 antibodies. The SUMOylation profile of a *Salmonella* infected intestinal epithelial cell was significantly lowered compared to that of uninfected cells (Fig. 1). This led to the hypothesis that *Salmonella* mediated alteration of host SUMOylation machinery could be a crucial strategy employed by this pathogen to manipulate host cells. Using a multipronged approach, we have initiated investigations to gain deeper understanding of this phenomenon and its implications. First, we sought to elucidate the host proteome that undergoes SUMOylation alteration by *Salmonella* during infection. This will comprise the infection specific SUMOylated proteome (SUMO-ome).

In order to gain better understanding of the phenomenon of SUMOylation alteration by *Salmonella*, we carried out time-course global SUMOylation profile assays. HCT8 cells were infected with wildtype strain SL1344. The cells were lysed (in the presence of various protease inhibitors and deSUMOlase inhibitors) at different time points (30 min, 1hr, 3hrs and 4 hrs) and immunoblotted for SUMO1, UBC9 - a key enzyme in SUMOylation reaction – and GAPDH, a housekeeping gene (Fig.2).

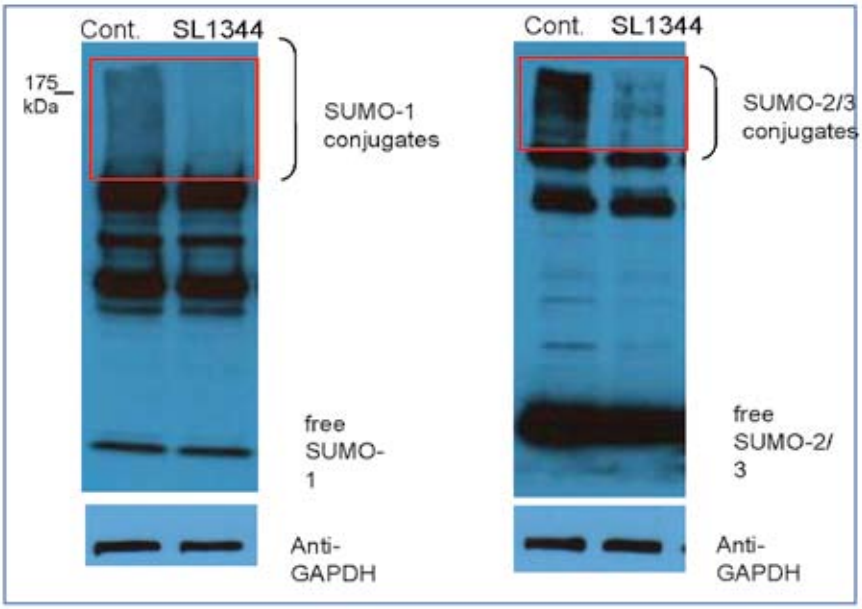


Fig.1: *Global SUMOylation profile assay*

It was evident that *Salmonella* infection leads to decrease in host SUMOylation, a process that begins at 1 hr post infection and reaches a maximum at 4 hrs post-infection (Fig. 2a). Concomitant decrease in the levels of UBC9 were also seen which further validates the mechanism (Fig. 2b). Levels of GAPDH remained unaltered throughout the experiment (Fig.2d). To further enrich the SUMO-modified proteins in this assay, we immunoprecipitated the lysates from above experiment using SUMO-1 antibodies followed by immunoblotting. Here too we observed severe reduction in host SUMOylation by *Salmonella* (Fig. 2d). It was important for us to understand the temporal dynamics of SUMO-proteome alteration before we could probe deeper into the mechanism of infected samples.

We next set out to identify SUMO1-proteome alterations during *Salmonella* infection. Formation of polySUMOylated chains occurs in case of SUMO2 and SUMO3 target proteins (hereafter referred to as SUMO2/3 proteome), while SUMO1 is almost always singly conjugated. Moreover the subset of target proteins conjugated by SUMO-1 is different from SUMO2 and SUMO3. In order to cover the entire SUMOome (comprising of SUMO1, SUMO2 and SUMO3) we have designed separate protocols for both these subsets.

To identify SUMO1-conjugated proteome, our protocol is a modification of a recent report [Tatham *et al, Nature Protocols* (2009) 4: 1363]. We have generated a His-SUMO expressing HCT8 cell line with expression of His-tagged SUMO1. This was required to bring the system physiologically closer to native relevance since excessive expression of SUMO1 could lead to non-specific target modifications. The cells were infected with SL1344 or left untreated, lysed in the presence of various protease

inhibitors and deSUMOlase inhibitors. Lysates were purified using Nickel affinity chromatography under denaturing conditions (using Guanidine- HCl and Urea). The immunoprecipitate complexes were then immunoblotted for SUMO1 to examine if the precipitation yields results similar to our previous experiments. We obtained the anticipated results. Separate aliquots of the lysates were trypsin-digested and subjected to high resolution mass-spectrometry (ESI5600 ABSciEX). We are currently in the process of standardizing the high resolution mass spectrometric experiments.

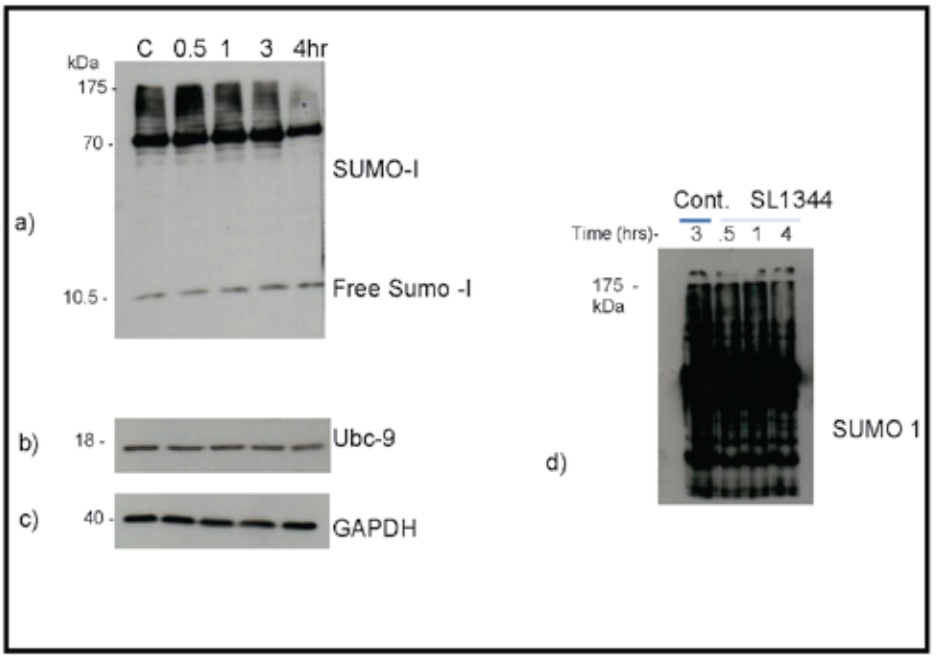


Fig. 2: *Time-course of global SUMOylation assay of SL1344 infected HCT8 cells Immunoblotted for (a) SUMO1, (b) UBC9, (c) GAPDH or immpunoprecipitaed (d) for SUMOylated proteins*

We have also begun investigation of *Salmonella* effectors mediating SUMOylation alteration during infection. Our aim was to identify and understand the mechanism engaged by *Salmonella* to alter host-SUMOylation. Using our global SUMOylation profile assay (Fig. 1) we have begun to compare the SUMOylation alteration as done by SL1344 (wildtype *Salmonella*) with that of various *Salmonella* mutants which are impaired for effector proteins or regulators of virulence proteins. Specifically we have generated mutations in virulence genes. Some of these mutants along with SL1344 were used to infect HCT8 cells for 3 hrs followed by immunoblotting for SUMO1, SUMO2/3 or GAPDH. We observed that in comparison to buffer control the SL1344 showed a decrease in overall SUMOylation (Fig. 3), while the mutant ssaV, a structural component of *Salmonella* pathogenicity island 2 (SPI2) , exhibited a reduced SUMOylation alteration in comparison to the wildtype bacteria. The SUMOylation profile of this mutant resembles more or less the buffer control indicating that the SPI2 loci might be involved in modulating the host-SUMOylation. However since SsaV encodes for a structural component of the injection apparatus, more experiments are required for deeper understanding of this mechanism employed by bacteria (discussed in future plans).

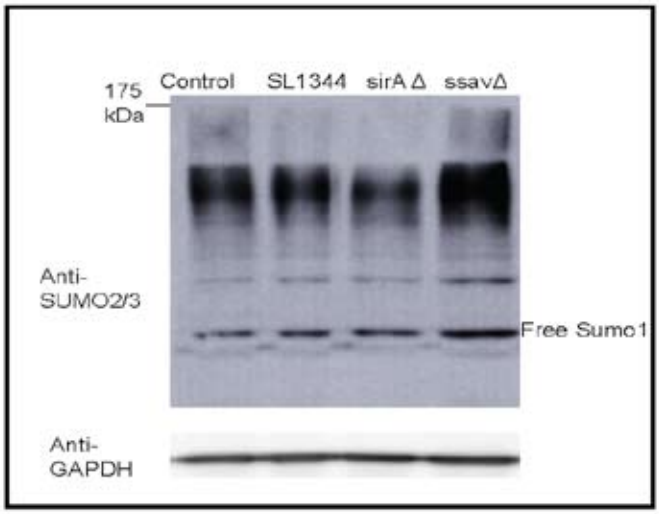


Fig. 4: *Comparison of global SUMOylation profiles of HCT8 cells infected with either SL1344 (wildtype) or various mutant Salmonella*

Future plans

1. Standardization of mass-spectrometric analysis for identification of SUMO1 modified proteome
2. Identification of SUMO2/3 proteome will be carried out using a protocol that exploits the poly-SUMO binding function of human ring finger protein RNF4. We are already in the process of generating the reagents for these experiments.
3. As an alternate approach we are also using Yeast 2 hybrid based assays to identify the SUMO-modified proteome in infected and untreated samples.
4. Once the proteins undergoing SUMOylation are identified they will be validated using co-immunoprecipitation and bioinformatics methods. These will be our candidate proteins.
5. The candidate proteins will be tested for their role in *Salmonella* infection as well as inflammation (by carrying out invasion and inflammation assays in knock/down cell line as we have earlier done. If knock-out mouse is available for the given gene, we will examine the effect of these genes using our *in vivo* colitis model.
6. The connection of the identified protein(s) for their association with the machinery of auto immune disorders (inflammatory bowel disease) in humans will also be investigated in collaboration with Dr. Vineet Ahuja (Department of Gastroenterology, AIIMS, New Delhi).

Publications

Original peer-reviewed articles

1. §Srikanth CV, Mercado-Lubo R, Hallstrom K and McCormick BA (2011) Salmonella effector proteins and host cell responses. *Cell Mol Life Sci*, 68:3687-97.

§ Workdone elsewhere, published during the reporting year.

Pathophysiology of Hemolysis and Thrombosis

Principal Investigator Prasenjit Guchhait, Ph D

Junior Research Fellow Gowtham Kumar Annarapu

Theme of Research

Intravascular hemolysis causes many vascular dysfunctions in patients with hemolytic disorders including sickle cell disease, thalassemia, PNH, HUS and malaria. Lysis of RBCs releases excessive extracellular hemoglobin (Hb) in plasma that interacts with many plasma proteins/factors to promote pathophysiological complications such as blood vessel occlusion (causing strokes, heart attack) in these patients.

Objectives

To understand the hemolysis-induced thrombosis (major cause of blood vessel occlusion) in hemolytic diseases, we will study the detailed interaction of extracellular Hb with prothrombotic plasma proteins/factors in patients. We have recently shown [Zhou *et al* 2009*,Thromb Haemost*101 (6):1070 - 77; Zhou et al 2010, *Semin Thromb Hemost*36 (1): 71 – 81]; Zhou *et al* 2011a,*Thromb Haemost*105 (6):1046 – 52; 2011b, *Anemia*2011: 918916; Guchhait *et al* 2012 (submitted)] that the extracellular Hb crucially regulates the activity of the plasma glycoprotein, von Willebrand factor (VWF) that serves the normal hemostatic functions by initiating platelet thrombus formation at the site of injury and stops bleeding.

The goal of our study is to identify: 1) more details of extracellular Hb interactions with VWF including the binding kinetics, stoichiometry and their precise binding sites using the recombinant polypeptides of VWF and Hb; 2) Determine the molecular mechanism (for) how extracellular Hb activates VWF to bind GP1bα on platelet surface to promote thrombus formation; 3) Determine the pathophysiological mechanisms (of) how extracellular Hb promotes the events of thrombosis in hemolytic conditions and 4) Determine the *in vivo* correlation between the hemolysis (extracellular Hb)and prothrombotic factors (including hyper-reactive VWF) in plasma of the hemolytic patients.

Progress during 2011-12

As the extension of my research work from the Baylor College of Medicine, I have started reproducing the data in this new establishment at RCB. We are in the process of developing and purifying recombinant polypeptides of different domains of VWF (such as A1, A2, A3). Our recent work shows that Hb binds to VWF-A2 domain [Zhou et al 2009, *Thromb Haemost*101 (6):1070 – 77], as well as VWF-A1 [Guchhait et al 2012 (submitted)]. The details of Hb binding to VWF-A1 or -A2and their binding interactions are as yet unknown. We made some progress towards determining the binding kinetics of Hb to A2 using the surface plasmon resonance (SPR) technique. The increasing concentration (7.3 -29.4 μM) of human erythrocyte purified HbA (normal Hb;Sigma, India) bound to the recombinant VWF-A2 coated CM5 chip in dose-dependent manner, under the flow condition (30μl/min) at 25^0C(y-axis represents response units, RU; x-axis for time in sec).

We are in the process of/ have made progress towards measuring the kinetics of HbA binding to VWF-A1 or VWF-A3 domain (control, since our study shows that Hb does not binds to VWF-A3) using the SPR method.

We are in the process of developing different deletion mutations of VWF-A1 and VWF-A2 domains to determine the precise binding site for HbA.

We have established the parallel flow chamber assay system in our RCB facility to perform *in vitro* studies. In the presence of HbA, VWF actively bound platelet GP1bα and promoted the adhesion of platelets on fibrinogen-coated surface under flow shear conditions. On the other hand, platelet adhesion is significantly less in the absence of HbA; figure is not shown).We started reproducing our data, showing that increasing concentration of extracellular Hb promotes VWF-mediated platelet thrombus formation on immobilized surfaces of sub-endothelium matrices (such as fibrinogen, collagen) or endothelium cell monolayer. Using this parallel flow chamber assay we will perform more experiments to understand the domain(s) and conformation(s) of VWF that binds maximally to platelets GP1bα in the presence of extracellular Hb under flow shear conditions (similar to flow shear conditions that exist indifferent blood vessels).

Future plans

To understand the detail of Hb-VWF interactions we will further examine the binding kinetics of different types or mutants (natural) of Hb (HbA_0, HbA_1, HbS, HbF and HbC) with VWF-A1,-A2, -A3, -A1A2A3 (75 KD) or VWF-full length (250 KD). We will also examine whether the monomer (α or β globin), dimer (αβ) or tetramer $(\alpha\beta)_2$ of Hb binds maximally to VWF.

To test our hypothesis that extracellular Hb-induces VWF binding to platelet GP1bα and thrombus formation, we will perform *in vivo* experiments using mice models of intravascular hemolysis (such as $ThCD59^{RBC}$mice) or hemolytic disorders (such as sickle cell anemia, thalassemia, malaria). We will collaborate with Dr. Rolando Rumbaut, Baylor College of Medicine, USA, to perform intravital microscopy experiments in mice. Alternately, we will develop *ex vivo* experiments using the parallel flow chamber assay, where we will grow mouse endothelium cells (collected from specific mice, i.e. $ThCD59^{RBC}$ mice) on culture plate (which will be used as the base for flow chamber experiment), and will perfuse whole blood (collected from $ThCD59^{RBC}$ mice) on the endothelium.

In addition, we will perform study to establish a detailed correlation between the extracellular Hb and prothrombotic factors (including hyper-reactive VWF) in plasma of the hemolytic patients such as in SCD, thalassemia and malaria.

To translate our molecular and genomic findings on diseases to clinical practice, we will develop anti-thrombotic and anti-adhesive therapeutics and test them *in vivo* in mice models. We will use our knowledge and expertise[Guchhait et al 2004, *J Immunol Meth* 295: 129–37; Guchhait et al 2008, *Thromb Haemost* 99: 552–57] to develop anti-thrombotic scFv (single chain antibody from phage library), monoclonal, polyclonal antibodies or small polypeptide, and will test *in vivo* in mice.

Publications

Review/Proceedings

1. §Zhou Z, Yee D and Guchhait P (2012). Molecular link between intravascular hemolysis and vascular occlusion in sickle cell disease. Curr *Vasc Pharmacol* (in press)

2. Tiwari R, Kumar G and Guchhait P (2012). Pathophysiological consequences of hemolysis in sickle cell disease. in Sickle *Cell Disease*; Nova Science Publishers, Inc *(in press)*

§ Workdone elsewhere, published during the reporting year.

Intrinsic Signals that Regulate Skeletal Muscle Structure and Function

(New Proposal)

Principal Investigator Sam Jacob Mathew, Ph D

Theme of Research

The skeletal muscle is one of the largest tissues in animals, responsible for mobility, posture, support and maintenance of body temperature. Research focuses on understanding how the mammalian skeletal muscle develops in the embryo, is maintained in the adult, and how the skeletal muscle regenerates during injury or disease.

Objectives

In order to study mammalian skeletal muscle development, differentiation, maintenance and regeneration, a family of muscle specific genes called Myosin heavy chains (MyHCs) are investigated. MyHCs are critical to skeletal and heart muscle structure and function. There are multiple MyHCs and their expression is temporally and spatially restricted based on their functional relevance. The major objective is to understand the functional role of one of these MyHC genes called MyHCembryonic (MyHCemb) in mice, by generating gene targeted mice where MyHCemb can be deleted in a temporally restricted manner during specific developmental myogenic phases and in injury. This should lead to deciphering the function of MyHCemb in skeletal muscle development, differentiation, maintenance and regeneration, understanding the consequences of losing the function of MyHCemb in skeletal and heart muscle, and how this leads to diseases. It has been planned to also investigate the role of MyHCemb *in vitro* by siRNA mediated knockdown in myogenic C2C12 cells.

Future plans

In the future, the studies on MyHCemb detailed above would be extended to an enhancer analysis for identifying the cis-regulatory sequences critical for its expression *in vitro* and *in vivo*, during development and injury. Special focus will be on the role of Wnt signaling in regulating MyHCemb expression. Mutations in MyHCemb have been linked to congenital contracture diseases like Freeman-Sheldon syndrome in humans. Understanding the genetic basis of the specific mutations in MyHCemb that cause such syndromes, identify the mechanistic basis of such diseases and generate possible diagnostic and therapeutic tools are the areas of interest. Studies on MyHCemb will be expanded to include other MyHCs, mutations in all of which have been reported to cause a multitude of muscle and heart associated diseases such as contractures and myopathies. Specific aim would be to generate alleles for each of the 7 skeletal muscle MyHCs in mice and study the independent role of each of the MyHCs in skeletal and heart muscle development, differentiation, homeostasis, regeneration and disease. In cases where redundancy between specific MyHCs is an issue, generation of double-targeted alleles will also be explored so that the redundant MyHCs can be deleted together. Another interesting question planned to be investigated is on epigenetic modifications that are characteristic to MyHCs and how that affects their function. By studying the independent and combined roles of each of the MyHCs and their regulation, it is believed that important insights into skeletal and cardiac muscle development, function, maintenance and disease may be gained.

Profiles of Faculty Members joining during 2011-2012

Sam Jacob Mathew

Postdoc: **University of Utah, Salt Lake City, Utah, USA**

PhD 2006: **University of Cologne, Germany**

Research Interests:

Research focused investigating the MyHCs in the heart and the skeletal muscle with respect to their function, regulation and evolution. These studies will help understanding how proper skeletal and cardiac muscle fiber differentiation occurs during development and in the adult as well as provide valuable insights into the functional importance of MyHCs. Specifically, the investigations that are proposed to be done using tissue samples from human patients with congenital contracture syndromes will not only provide a mechanistic basis to our understanding of these syndromes but could also lead to the identification of new genes that are involved in causing this class of diseases as well as aid in developing new therapeutic diagnostic and treatment options for such patients. Understanding the evolution of the MyHCs should inform us about the functional diversification of the MyHCs and the metabolic properties of each of them. This in turn will be important in understanding the metabolic role of the skeletal muscle and how it regulates whole body metabolism. The innovations in gene targeting will help in deleting multiple genes in the same animal at the same time, even if the genes are closely linked, thereby addressing the problem of functional redundancy in model organism studies.

Selected Publications:

1. Mathew, S. J., M. Rembold, and M. Leptin. 2011. A role for Traf4 in polarizing adherens junctions as a prerequisite for efficient cell shape changes. *Mol. Cell. Biol.* 2011, 31:4978.

2. Murphy, M. M., J. A. Lawson, S. J. Mathew, D. A. Hutcheson, and G. Kardon. 2011. Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. Development 138:3625-3637.

3. Mathew, S. J., J. M. Hansen, A. J. Merrell, M. M. Murphy, J. A. Lawson, D. A. Hutcheson, M. S. Hansen, M. Angus-Hill, and G. Kardon. 2011. Connective tissue fibroblasts and Tcf4 regulate myogenesis. Development 138:371-384. .

4. Mathew, S. J., D. Haubert, M. Kronke, and M. Leptin. 2009. Looking beyond death: a morphogenetic role for the TNF signalling pathway. J Cell Sci 122:1939-1946.

5. Mathew, S. J., S. Kerridge, and M. Leptin. 2009. A small genomic region containing several loci required for gastrulation in Drosophila. PLoS One 4:e7437

Scientific Activities and Achievements

RCB Colloquia

RCB organized three scientific colloquia inviting outstanding scientists from three continents namely Dr. Ravi Acharya, University of Bath, UK; Dr. Richard Jefferson, Queensland University of Technology, Australia and Prof. Sally Ward Ober, UT Southwestern Medical Centre, USA.

Dr. Acharya is Professor of structural molecular biology at University of Bath and focuses on structure-function studies of molecules involved in disease processes. His team's interests range from extremophile enzyme stability and catalysis to the elemental processes involved in inflammation, immunity (innate and adaptive) and neurodegenerative diseases. During the colloquium Dr. Acharya shared his views on where structural biology is now, and what its future holds with specific emphasis on drug discovery.

Dr. Jefferson, a molecular biologist is also very much known for his expertise on intellectual property. As CEO, Cambia, the BiOS Initiative, which he founded in 2005, is active in the promotion of open source biology of a similar philosophy to that in informatics. During his recent visit to RCB, he delivered a colloquium talk on 'Disruption by Design: Biological Open Source, its origins and its future'. He described both the historical origins of biological open source innovation, and his own journey in developing it further in the context of modern biotechnology. He also described the efforts he put-in to create an open, patent-based commons of tools for agricultural biotechnology, visualizing the barriers faced and the lessons learnt.

Prof. Ober, who is Paul and Betty Meek-FINA Professor of Molecular Immunology at the UT Southwestern Medical Centre, USA works in the field of molecular immunology with her research directed towards taking an interdisciplinary approach to generate effective therapeutics for autoimmunity and cancer. This approach involves, among other things, development of engineered antibodies that are altered in their binding properties for FcRn and, as such, can modulate FcRn function. At the colloquium she spoke on 'FcRn as a therapeutic target: from subcellular behavior to in vivo studies in mice' highlighting the importance of understanding how FcRn performs its function as a salvage receptor within cells and at the whole body level.

Workshops conducted by RCB

1. RCB organized a workshop on **Platforms for Molecular Cross-talks in Modern Biology** during 4-9 June 2012. 25 Students/post-doctoral fellows from diverse places and disciplines participated. This workshop showcased in vivo as well as in vitro approaches to detect and dissect molecular interactions in cellular systems. Expert faculty practicing these approaches in their on-going research delivered lectures and provided didactic training using state-of-art equipments. Participants gained research- based training and in-depth knowledge of the platforms employed in understanding molecular communications in biology.

2. Another workshop on **Eukaryotic Model Organisms** was organized during October 12-16, 2012. This workshop highlighted three popular model systems - yeasts, a nematode worm and the fruit fly. Experts imparted theoretical knowledge and practical training to showcase the power and versatility of these organisms, which have enabled path-breaking discoveries in basic biology and continue to revolutionize advances in biotechnology and medicine. 25 participants from institutions/ universities across India benefited from the training imparted in this workshop.

Workshop on Platforms for Molecular Cross-talks in Modern Biology (June 4-9, 2012)

Workshop on Eukaryotic Model Organisms (October 12-16, 2012)

Seminars delivered by visiting scientists at RCB

Speaker	Title	Date
E Sally Ward Ober, PhD University of Texas Southwestern Medical Center Dallas, USA	FcRn as a therapeutic target: from subcellular behavior to in vivo studies in mice	September 28, 2012
Richard Anthony Jefferson, PhD CEO Cambia & Queensland University of Technology, Australia	Disruption by Design: Biological Open Source, its Origins and its Future	September 27, 2012
K Ravi Acharya, PhD University of Bath, UK	Structural Molecular Biology: A Cutting Edge Science for Drug Discovery	September 26, 2012
Chinmoy Patra, PhD Max Planck Institute for Heart and Lung Research, Germany	Identification of novel regulators in cardiovascular development	September 17, 2012
Vineet Gaur, PhD Department of Chemistry and Biochemistry,The Ohio State University, OH	Sugar-selectivity and stereoselectivity among DNA polymerase	September 14, 2012
Nirala Ramchiary, PhD Assistant Professor, University of Gauhati, Gauhati	Integrating Genetics and Genomics in Brassica Breeding	August 27, 2012
Sai Krishnaveni Manda, PhD University of Southern California, Los Angeles	Epithelial-specific TGF-ß signaling of the lung: Evidence for an epithelial role in Pulmonary Fibrosis	August 17, 2012
Rohini Garg, PhD National Institute of Plant Genome Research, New Delhi	Genomics to epigenomics: an integrated approach to understand stress adaptation in plants	August 16, 2012
Swasti Raychaudhuri, PhD Max Planck Institute of Biochemistry Munich, Germany	A Complex Protein Network Regulates the Cytosolic Stress Response - Specific Role of Proteasome	August 13, 2012
Jeetender Chugh, PhD University of Michigan, Ann Arbor, MI, USA	Visualizing Transient Structures in A-site RNA of Ribosome	July 27, 2012
Koyeli Mapa, MBBS PhD Institute of Genomics and Integrative Biology, New Delhi	Information encoded by non-native states drive substrate-chaperone pairing	July 23, 2012
Arvind Sahu, PhD National Centre for Cell Science, Pune	Studies on complement evasion in poxviruses provide insight into their host tropism	June 08, 2012

Utpal Tatu, PhD Indian Institute of Science, Bangalore	Mass spectrometry based identification of trans-splicing in Giardia lamblia Hsp 90	June 07, 2012
Subhra Chakraborty, PhD National Institute of Plant Genome Research, New Delhi	Comparative proteomics display seed protein regulated dynamic protein network in plant	June 06, 2012
Sudipto Maiti, PhD Department of Chemical Sciences, Tata Institute of Fundamental Research, Mumbai	Protein misfolding versus aggregation: following the early events with an ultra-sensitive fluorescence tool	June 05, 2012
Sanjeev Galande, PhD Indian Institute of Science Education and Research,Pune	Signaling to chromatin: Tale of a genome organizer	June 04, 2012
Abhik Saha, PhD Perelman School of Medicine University of Pennsylvania, Philadelphia, USA	Targeting Apoptosis-Autophagy Network in Virus Associated Human Cancers - A Therapeutic Approach	June 01, 2012
Atanu Maiti, PhD University of Maryland School of Medicine, Maryland, USA	Mechanism of Human TDG in maintaining genetic and epigenetic integrity	May 24, 2012
Rahul Purwar, PhD Harvard Medical School, Boston, USA	Engineering robust immune system to cure cancer	May 22, 2012
Debabrata Mandal, PhD Massachusetts Institute of Technology, Cambridge MA	Transfer RNA modifications and their role in translational fidelity	April 30, 2012
Jagpreet S. Nanda, PhD Johns Hopkins University School of Medicine, Baltimore	Gene to Protein...Good beginning makes a good ending	April 12, 2012
Padubidri Shivaprasad, PhD University of Cambridge Cambridge, UK	Uncovering the hidden potential: Epigenetic Influence on hybrid phenotypes	March 26, 2012
Sudipto Saha, PhD Case Western Reserve University Cleveland, Ohio, USA	Systematic Discovery of Protein Networks using Computational Proteomics	March 14, 2012
Gagandeep Kaur Gahlay, PhD University of Wyoming, Laramie USA	Evolving Paradigms in Sperm-Egg Recognition	February 28, 2012
Mary Munson, PhD UMass Medical School Worcester MA, USA	Molecular Architecture of the Exocyst Complex and its Function in Exocytosis	February 27, 2012

Sreedhara R Voleti, PhD Institute of Life Sciences Hyderabad,	Synergistic Applications of In Silico Studies and Crystallography in Drug Design - Case Studies	February 23, 2012
Santanu Banerjee, PhD University of Pennsylvania, Philadelphia PA	Genetic dissection of neural crest cell migration	February 07, 2012
Athulaprabha Murthi, PhD Director, IndiaBioscience Bangalore	The IndiaBioscience Initiative	February 06, 2012
Alok Sharma, PhD The Rockefeller University, New York, USA	Structure, Function and Dynamics of three-fold symmetric plant lectins	February 06, 2012
Sam Jacob Mathew, PhD University of Utah Salt Lake City, Utah, USA	Skeletal muscle building: the ins and outs	February 06, 2012
Rajakumara Eerappa, PhD Memorial Sloan-Kettering Cancer Center, New York, USA	Structural and Biochemical Basis for Methylated DNA and Histone Codes Read-out and Interpretation by Chromatin Associated Modular Proteins	February 01, 2012
Purusharth Rajyaguru, PhD University of Arizona, Tucson Arizona	To translate, remain silent or get destroyed: How are mRNA fate decisions made?	January 31, 2012
Dhirendra K Simanshu, PhD Memorial Sloan-Kettering Cancer Center, New York, USA	Molecular machineries involved in trafficking of signaling lipids and small RNA-mediated gene silencing	January 30, 2012
Soumen Kanti Manna, PhD National Cancer Institute, National Institutes of Health, Bethesda, MD, USA	Metabolomics: A Promising Approach for Diagnostic and Mechanistic Investigation	January 27, 2012
Amar Nath Gupta, PhD University of Alberta, Edmonton, AB, Canada	Reconstruction of Free Energy Landscape for Native Folding of the Prion Protein from Single-Molecule Force Spectroscopy using Optical Tweezers	January 18, 2012
Uma Rao, PhD Indian Agricultural Research Institute, New Delhi	Plant nematode genomic resources paving the way for new generation management tools	January 18, 2012
Prashant Kodgire, PhD University of Chicago, Chicago IL USA	Chromatin, transcription and somatic hypermutation of immunoglobulin genes	January 17, 2012

Chandra Prakash Chaturvedi, PhD The Sprott Center for Stem Cell Research, Ottawa Health Research Institute, Ottawa, ON, Canada	The functional role of histone methyltransferase G9a in regulating gene expression program in adult erythroid cells	January 12, 2012
Jeet Kalia, PhD National Institutes of Health Bethesda, MD, U.S.A.	Chemistry, Biology and the Interface	December 29, 2011
Radha Devi Chauhan, PhD The Rockefeller University New York, USA	Insight into Structure and Versatility of the Transport Channel of the Nuclear Pore Complex	December 29, 2011
Anand Srivastava, PhD Institut National de Transfusion Sanguine, Inserm/Universite Paris, Diderot Paris, France	Malaria: Insight into the mechanism of Invasion and Cytoadherance	December 28, 2011
Shireesh Srivastava, PhD National Institute of Alcohol Abuse and Alcoholism, NIH, USA	Systems biology and biochemical investigations of (fat) metabolism	December 26, 2011
Uma Chandra Mouli Natchu, MD Pediatric Biology Centre, Translational Health Science and Technology Institute, Gurgaon	Beyond 2x2 tables - data rich discovery in public health and clinical research	December 22, 2011
Rahul Roy, PhD Harvard University Cambridge, MA, USA	Single molecule Biology: a new perspective for biology	December 21, 2011
Deepak T Nair, PhD National Centre for Biological Sciences, Bangalore	Sub-site spacing in operator sequences influences mode of interaction of AraR-NTD with DNA	December 8, 2011
Rajeev Kumar, PhD Institut de Genetique Humaine CNRS Montpellier, France	Conservation of mouse Rec114 and Mei4 in programmed breaking of the genome	December 7, 2011
Susmit Basu, PhD Bayer Material Science, CAT Catalytic Center, Aachen, Germany	Enabling Catalysis Technology for Sustainable Production of consumable polymer	December 7, 2011
Shashi Bhushan Pandit, PhD Genoscope, CEA Evry cedex, France	Protein tertiary structure prediction in the post-genomic era	November 25, 2011
Rajan Gogna, PhD Ohio State University, USA	Role of Oxygen and Flower Code in Cancer and Cardiovascular Diseases	November 21, 2011

Samrat Mukhopadhyay, PhD Indian Institute of Science Education and Research, Mohali	How do Proteins Fold, Misfold and Aggregate?	November 18, 2011
Ranabir Das, PhD National Cancer Institute- Frederick, USA	“What is on your backside?” Unique ‘backside’ interactions between an ubiquitin-conjugating enzyme and ubiquitin-ligase regulate ubiquitylation	November 14, 2011
Amit Tuli, PhD Harvard Medical School (HMS) / Brigham and Women’s Hospital (BWH) Boston, MA, USA	“The Small Arf-like GTPase Arl8b Regulate Lytic Granule Polarization and Cell-Mediated Cytotoxicity in Natural Killer Cells”	November 8, 2011
Pabitra B. Chatterjee, PhD Colorado State University, Fort Collins, USA	Taking coordination chemistry into nano-confinement: A journey in vanadium chemistry	November 8, 2011
Ravi Jain, PhD Carleton University, Ottawa	Managing Innovation in Life Sciences	October 25, 2011
Ashok Venkitaraman, PhD MRC, Cambridge, UK	Translational Sciences and Clinical Application	September 16, 2011
Partha P. Bera, PhD NASA Ames Research Center	Application of Quantum Mechanics in Atmospheric Chemistry, and Radiation Chemistry of Biomolecules	August 24, 2011

Lectures delivered / Conferences attended/ Visits abroad

Dr. Dinakar M. Salunke

1. Delivered an invited lecture on 'Structural biology of plant seed proteome' at National Crystallography Seminar, Osmania University, Hyderabad, 26 November 2011

2. Delivered an invited lecture on 'Genesis & programmes of the Regional Centre for Biotechnology in India under the auspices of UNESCO' at Conference on Natural Products, 2012, Kathmandu, 18-20 April, 2012

3. Delivered a keynote address entitled 'New paradigms in antibody specificity: structural biology of immune recognition' at Venkateswara College, New Delhi 14 May, 2012

4. Delivered an invited lecture entitled 'Molecular mimicry and ligand specificity: experiences from immunological investigations' at the Indian Immunology Society - Odisha Chapter: 10-11 August 2012

5. Delivered a colloquium talk entitled 'Revisiting tenets of specificity and recognition in immune system' at Indian Institute of Science Education & Research, Mohali, 3 October, 2012

Dr. Prasenjit Guchhait

1. 53rd Conference of the American Society of Hematology 2011, San Diego, USA, 10- 13 December, 2011

Dr. Sivaram V.S. Mylavarapu

1. Attended the AACR conference on "New Horizons on Cancer Research" held at Gurgaon from 13-16 December, 2011

Dr. Avinash Bajaj

1. Delivered an invited lecture on 'Chemistry and Biology of Cancer' in KVPY Summer Program at Indian Institute of Science Education and Research, Bhopal, 15 June 2012.

2. Delivered an invited lecture on 'Engineering of Nano-therapeutics for Cancer Medicine' in AICTE-sponsored National Seminar on Recent Developments from Drug Discovery to Drug Delivery at Lord Shiva College of Pharmacy, Sirsa, Haryana during 28-29 April, 2012.

3. Delivered a keynote address on 'Why to Choose a Career in Science' in UGC-sponsored National Level Seminar "Green Chemistry" at Dept. of Chemistry, GHG Khalsa College, Ludhiana, 24 February 2012.

4. Delivered an invited talk on 'Exploiting Cancer Chemistry for Novel Therapeutics' in Prof. Ram Chand Paul National Symposium on Frontiers in Chemical Sciences at Panjab University, Chandigarh, 25 February 2012.

5. Delivered an invited lecture entitled 'Engineering of Nanomaterials for Cancer Cell Detection and Therapy' in 5th Symposium on 'The Frontiers in Molecular Medicine' at JNU, New Delhi during 17-18 February 2012.

6. Delivered an invited lecture on 'Bridging the Basic and Translational Research using Nanotechnology' in 4th Annual Biotechnology & Molecular Medicine Symposium, Rohtak, 30 November 2011.

7. Attended the 31th Annual convention of Indian Association for Cancer Research at ACTREC, Mumbai during 6-9 Janurary 2012.

Dr. K. Vengadesan

1. Delivered an invited lecture entitled "A structural model for Group B *Streptococcus pili* and its role in pathogenesis" at UGC-Resource Networking National Symposium on 'Microbes in Health and Agriculture', School of Life Sciences, Jawaharlal Nehru University, New Delhi, 12-13 March, 2012.

2. Delivered a talk entitled "Structural Analysis of Adhesive Tip pilin, GBS104 from *Group B Streptococcus*" at Annual Meeting of the Indian Biophysical Society, Centre of Advanced study in Crystallography and Biophysics, University of Madras, Chennai, 19-21 January, 2012.

3. Participated in 5th International symposium on "Recent Trends in Macromolecular Structure and Function (ISRTMSF 2012)", at Centre of Advanced study in Crystallography and Biophysics, University of Madras, Chennai during 23-25 January, 2012.

4. Participated in the International Symposium, "Accelerating India's Response to Research for a Preventive HIV Vaccine", New Delhi organized by Ministry of Science and Technology, Ministry of Health and Family Welfare, Govt of India during 13-14 August, 2012.

5. Visited Synchrotron X-ray beam line (BM14) of the European Synchrotron Radiation Facility (ESRF) at Grenoble, France, during 21-24 July, 2012 with travel support from Indian consortium of macromolecular Crystallography (funded by Department of Biotechnology, Government of India).

Dr. Chittur V. Srikanth

1. Delivered Lecture on "Microbiology research for exploring nature" at Acharya Narendra Dev College, University of Delhi South Campus on 31st August 2012

2. Attended International Symposium on Accelerating India's Response to Research for a preventive HIV vaccine organized by Ministry of Science and Technology, Ministry of Health and Family Welfare, Govt. of India during 13-14 August, 2012

Membership of professional/ Academic bodies/ Editorial Boards

Dr. Dinakar M. Salunke

1. Member, Governing Council, National Brain Research Centre, Manesar
2. Member, Governing Body, National Institute of Plant Genome Research, New Delhi
3. Member, Governing Body, Translational Health Science & Technology Institute, Gurgaon
4. Member, Commission on Biological Macromolecules, International Union of Crystallography
5. Consultant, Commission on Synchrotron Radiation, International Union of Crystallography
6. Member, Council of Scientific Advisers, International Centre for Genetic Engineering & Biotechnology
7. Member, Scientific Advisory Committee, Bose Institute, Kolkata
8. Member, Scientific Advisory Committee, National Brain Research Centre, Manesar
9. Member, Scientific Advisory Committee for Biosciences and Bioengineering Group, IIT Indore
10. Chairman, INSA National Committee for International Union of Crystallography
11. Member of Expert Committee on Fund for Improvement of S&T Infrastructure in Higher Educational Institutions (FIST), Department of Science & Technology
12. Member, Apex Committee, Biotechnology Industry Partnership Programme
13. Member, Finance Committee, National Brain Research Centre, Manesar
14. Member, INSA Sectional Committee on Cell and Biomolecular Sciences

Dr. Prasenjit Guchhait

1. Member of the Editorial Board for the Journal of Hypertension and Cardiology, USA.
2. Member of the Editorial Board for the World Journal of Hypertension, China.
3. Active member of the American Society of Hematology.
4. Professional member of the American Heart Association.
5. Associate Member of the Sigma Xi, the Scientific Research Society, USA.
6. Professional Member of North American Thrombosis Forum.
7. Associate member of the Dun L Duncan Cancer Center, Baylor College of Medicine, USA.
8. Member of the Association of Scientists of Indian Origin in America.

9. Member of the Pineal Study Group, India.
10. Member of the Society for Reproductive Biology and Comparative Endocrinology, India.

Dr. Avinash Bajaj

1. Life Membership: Proteomics Society of India
2. Life Membership: Chemical Society of India
3. Life Membership: Society of Biological Chemists of India

Distinctions, Honours and Awards

Dr. Dinakar M Salunke

1. Elected Vice President, Indian National Science Academy

Dr. Chittur V. Srikanth

1. Awarded India Alliance Wellcome Trust intermediate fellowship for a period of 5 years w.e.f. June 1, 2012.

Infrastructure development

Administrative Activities

During the year ending 31.3.2012, the position of staffing in the Centre was streamlined with the recruitment of staff with expertise in areas of Finance, Administration and Stores & Purchase. The processes & procedures for conduct of the administrative activities at the Centre were consolidated to enhance the functioning of the centre

Automation of the selected administrative functions was undertaken in consultation with the National Informatics Centre (NIC) cell of the Department of Biotechnology. E-governance web application was developed and implemented which led to operational flexibility and transparency. High speed Internet backbone through the National Knowledge Network was established. Video-conferencing connectivity was actively used to enhance coordination with DBT and other institutions. The Centre also established IT networking facility at the interim campus.

Interim Laboratories in NCR, Gurgaon

The Interim Laboratories at 180, Udyog Vihar, Phase I, Gurgaon (NCR) and research activities started during the period 2010 -11 were further enriched during the period under report. The Laboratories were furnished with new equipments, instruments and facilities like Advanced Proteomics Facility (MASS SPECTROMETRY), Surface Plasmon Resonance, ATOMIC FORCE MICROSCOPY –(BIO-AFM), Protein/Peptide Sequencer, FPLC protein purification system, Dispenser 4 Crystallisation, CD Spectropolarimeter, Flow Cytometer, Workstations (8 Nos.), Isothermal Titration Calorimeter, Floor Ultra Centrifuge, Fluroscence Microscope, Differential Scanning Calorimeter, CCD imager, Stackable incubator shaker, Shaker incubator, -80 Deep Freezer, High capacity Refrigerated Centrifuge, Real Time PCR machine, Flash Chromatography System, Automated flash chromatography, 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 costing above Rs. 5.00 lacs. An NMR Spectrometer has also been ordered during the period which is likely to be delivered and installed soon.

Permanent Campus of the NCR-BSC Project at Faridabad

The contract for civil, electric & HVAC work was awarded to M/s Odeon Builders P. Ltd. at a contract value of Rs.105.14 Crores & for elevator works M/s ThyssenKrupp elevator (I) P. Ltd. for Rs.4.0 Crores. The civil works started in July 2011. Overall progress of over 65% has been achieved as on date. Most of the RCC work, brick work and plastering have been completed. Two DG Sets, HT/LT panels and chilling system for air conditioning have been installed. One sample laboratory has been completed for design demonstration.

Atomic Force Microscope facility

Isothermal titration Calorimeter & Differential Scanning calorimeter facility

Cell Culture facility

A view of the construction at Faridabad Project site

Institutional Information

Committees of Regional Centre for Biotechnology

Board of Governors

1. Prof. M.K. Bhan (Chairman)
   Secretary, Department of Biotechnology,
   Ministry of Science and Technology, Govt. of India
   New Delhi

2. Mr. Shigeru Aoyagi
   Director & UNESCO representative to Bhutan,
   India, Maldives and Sri Lanka
   (UNESCO Office, New Delhi)

3. Prof. Akhilesh K. Tyagi
   Director,
   National Institute of Plant and Genome Research,
   New Delhi

Programme Advisory Committee

1. Dr. K. VijayRaghavan (Chairman)
   Director,
   National Centre for Biological Sciences,
   GKVK, Bangalore

2. Prof. Angelo Azzi
   JM USDA-HNRCA at Tufts University
   711 Washington ST.
   Boston MA 02111, USA,

3. Prof. Shankar Subramaniam
   Department of Bioengineering
   Department of Chemistry & Biochemistry
   University of California at San Diego, USA

4. Dr. G.B.Nair
   Executive Director,
   Translational Health Science & Technology Institute
   Gurgaon

5. Prof. Mriganka Sur
   Massachusetts Institute of Technology
   Head, Dept. Of Brain and Cognitive Science,
   2 Vassar St., 46-6227, Cambridge, MA 02139

6. Prof. Jeongbin Yim
School of Biological Sciences,
Seoul National University, Seoul,
151-742, Korea

7. Prof. K. Veluthambi
School of Biotechnology,
Madurai Kamaraj University, Madurai

8. Prof. R Venkata Rao
Vice Chancellor,
National Law School of India University,
Bangalore

9. Dr. Satyajit Rath
Senior Scientist
National Institute of Immunology,
New Delhi

10. Shri S. Sinha
Adviser
Dept. of Biotechnology
Ministry of Science and Technology
Govt. of India, New Delhi

11. Dr. K.V.S.Rao
Senior Scientist
International Centre for Genetic Engineering and Biotechnology,
New Delhi

12. Dr. Dinakar M. Salunke (Member Secretary)
Executive Director
Regional Centre for Biotechnology, Gurgaon

13. Dr. Ramesh V Sonti (Special Invitee)
Centre for Cellular & Molecular Biology
Habsiguda, Uppal Road
Hyderabad- 500007

14. Prof. Subrata Sinha (Special Invitee)
Director
National Brain Research Centre
NH-8, Nainwal Mode, Manesar-122050

15. Prof. E Sally Ward Ober (Special Invitee)
UT Southwestern Medical Center
5323 Harry Hines Boulevard
Dallas, Texas 75390

16. Dr. Richard Anthony Jefferson (Special Invitee)
    Director, Initiative for Open Innovation (IOI)
    Chief Executive Officer, Cambia
    Professor of Science, Technology & Law,
    Queensland University of Technology (QUT)
    Rogue, National ICT Australia (NICTA)

17. Dr. K. Ravi Acharya (Special Invitee)
    Department of Biology and Biochemistry
    University of Bath
    Claverton Down
    Bath BA2 7AY, UK

18. Prof. Akhilesh Kumar Tyagi (Special Invitee)
    Director
    National Institute of Plant Genome Research
    Aruna Asaf Ali Marg, New Delhi

Executive Committee

1. Dr. Dinakar M. Salunke (Chairman)
   Executive Director,
   Regional Centre for Biotechnology
   Gurgaon

2. Mr. Shigeru Aoyagi
   Director & UNESCO representative to Bhutan,
   India, Maldives and Sri Lanka
   (UNESCO Office, New Delhi)

3. Shri S. Sinha
   Adviser
   Dept. of Biotechnology
   Govt. of India
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4. Ms. Veena Ish
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   Ministry of Human Resource Development
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5. Shri A.R. Ghanshyam
   Joint Secretary,
   UNESCO Division,
   Ministry of External Affairs,
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Finance Sub-Committee

1. Dr. Dinakar M. Salunke (Chairman)
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2. Dr. G.B. Nair
   Executive Director
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4. Shri S. Sinha
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5. Dr. Satyajit Rath
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Staff of the Regional Centre

Scientific Faculty

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Dr. Sivaram V S Mylavarapu	Assistant Professor
Dr. Avinash Bajaj	Assistant Professor
Dr. Vengadesan Krishnan	Assistant Professor
Dr. Tushar Kanti Maiti	Assistant Professor
Dr. C.V.Srikanth	Assistant Professor
Dr. Sam Jacob Mathew	Assistant Professor
Dr. Falguni Sen	International Adjunct Faculty

Academic Management

Sh. B. D. Vashisth	Registrar
Sh. Deepak Kumar	Documentation Assistant
Ms. Vaishali Mangla	Documentation Assistant

Administration

Sh. Biju Mathew	Senior Manager (A&F)
Dr. Sanjay Kumar	Staff Officer to Executive Director
Sh. V.M.S. Gandhi	Administrative Officer
Sh. Sanjeev Kumar Rana	Jr. Management Assistant
Sh. Sudhir Kumar	Jr. Management Assistant
Sh. Kulwant Singh	Jr. Management Assistant

Engineering

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Technical

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Sh. Suraj Tewari	Technical Assistant
Sh. Vijay Kumar Jha	Technical Assistant
Ms. Vishakha Chaudhary	Technical Assistant
Sh. Atin Jaiswal	Technical Assistant
Sh. Ramesh Chandiramouli	Technical Assistant

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Sh. C.L. Raina, Consultant (Finance)
Sh. S.S. Budhwar, Consultant (Engineering)
Sh. Bansi Lal Shah, Jr. Consultant (Finance)
Sh. R.N. Chatterjee, Jr. Consultant (Admn.)

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