United Nations
Educational, Scientific and
Cultural Organization

REGIONAL CENTRE FOR BIOTECHNOLOGY

an institution of education, training and research

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

Annual Report
2012 – 2013

United Nations
Educational, Scientific and
Cultural Organization

REGIONAL CENTRE FOR BIOTECHNOLOGY

an institution of education, training and research

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

Cover inset - *New laboratories of RCB at the Biotech Science Cluster, Faridabad*

Annual Report
2012 – 2013

Contents

From the Executive Director's Desk

It has been a year of further growth and development at the Regional Centre for Biotechnology and I am indeed very pleased to bring this report while taking stock of where we stand today. As the construction of the laboratory building in the Faridabad campus is nearing completion, we have been busy building the infrastructure and research & academic programmes at the Gurgaon campus. In doing so, we have tried to cope with our own expectations.

We have accelerated our efforts in multidisciplinary facets of biotechnology while initiating new research programmes as well as the myriad academic activities. Our research towards engineering of nanomaterials for biomedical applications is focused on the systematic exploration of synthesis and activity profiles of bile acid amphiphiles on colon cancer cells. Both apoptotic and cell-proliferative behavior of these molecules is directly linked to the nature of their molecular architectures. Towards exploring the molecular mechanisms of infectious and idiopathic inflammation, Salmonella induced SUMO-proteome perturbations were observed. SUMOylation mechanism appeared to play a crucial role in infection as well as Salmonella-induced inflammation. To address the problem of thrombosis and hypercoagulation in hemolytic disorders, a series of in vitro and ex vivo experiments are underway to understand the molecular interactions among plasma cell-free hemoglobin, von Willebrand factor and platelet surface glycoproteins that lead to intravascular platelet clots formation and blood vessel blockage. To understand the intrinsic signals mediating skeletal muscle differentiation, myosin heavy chain gene function was investigated using in vitro and in vivo approaches.

Structural biological approaches are being explored towards addressing regulatory events in physiological processes. The work concerning the comparative structural proteomics of edible plant seeds under the allergy screen has provided interesting leads correlating the specific molecular properties with allergy-specific epitopes. In understanding the transition from metaphase to anaphase that is monitored by the spindle assembly checkpoint, differential contributions of the light intermediate chain subunit homologs of cytoplasmic dynein were analyzed in facilitating cell cycle progression. Towards understanding bacterial pilus architecture, assembly, and pili-mediated host interaction, high resolution x-ray diffraction data were collected and are being analysed for a backbone-pilin from probiotic Lactobacillus rhamnosus GG.

From the Executive Director's Desk

In understanding the role of BRCA1 associate protein 1(BAP1) in cancer, it has been shown that the mutations of BAP1 undergoes fibrillar aggregation which might be the possible cause of BAP1's cancer association.

Two new faculty members joined this year bringing the total strength of regular faculty to ten. The new members have initiated studies in the area of plant-pathogen interactions aimed towards understanding of trigger and signaling routes of effector-triggered immunity mediated by inositol-derivatives and to understand the mechanistic details of immune cell dysfunctions linked to complement regulatory protein deficiency.

The programme for mentoring young investigators has been very successful; we have been able to attract several meritorious young scientists immediately after their doctoral degrees to join RCB and conduct their research under faculty mentorship. Training activities are also continuing in terms of laboratory-based workshops, visiting students on study tours from within and outside India, and working of project trainees as part of the under-graduate and post-graduate curricula. Twenty research fellows working under the supervision of the RCB faculty are now enrolled for the Ph D programme at the Manipal University of which RCB is a recognized Research Centre. This has enabled nucleation towards multi-disciplinary academic programmes in Biotech Sciences as per the mandate. This will eventually be continued indigenously after the enactment of the RCB Bill 2011 under consideration in the Parliament of India.

RCB was conceived by the Govt. of India as an institution of education, training and research in biotechnology in India and in this region under the auspices of UNESCO as a category II Centre. It is well recognized that the presently underway biotechnology revolution cannot be restricted by national boundaries. There is remarkable advantage to work together, particularly for human resource development, with international organizations. In this context, UNESCO's association has been critical towards developing linkages with the centres of excellence globally and facilitating connectivity with other member states in the region.

The RCB faculty along with the young investigator awardees, research fellows and the research staff working in various extramural projects, have provided the required critical mass for the vibrant scientific ambience at the Centre. Combined with these, the dedicated technical and administrative staff have created institutional structures for facilitating fulfillment of the mandates of the Centre. Equally valuable is the support from the colleagues at the Department of Biotechnology, Ministry of Science and Technology, Govt of India and the concerned officials at UNESCO. Indeed, this is an exciting phase in the metamorphosis of RCB, an institution that aspires to be counted among the best in the world.

Dinakar M. Salunke
Executive Director

Mandate of the Centre

Mandate of the Regional Centre for Biotechnology is to provide a platform for biotechnology education, training and research at the interface of multiple disciplines. The programmes of the Centre are designed to create opportunities for students to engage in multi-disciplinary research where they learn biotech science while integrating engineering, medicine and natural sciences, to provide solutions for human and animal health, agriculture and environmental technologies. The vision is to produce human resource tailored to drive innovation in biotechnology, particularly in areas of new opportunities and also to fill talent gap in deficient areas.

The Centre shall be an institution of international importance for biotechnology education, training and research (and shall, in due course, be constituted as an autonomous body under an Act of the Parliament). The Centre is regarded as a "Category II Centre" in terms of the principles and guidelines for the establishment and functioning of UNESCO Institutes and Centres.

The Centre functions with following objectives:

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

Scientific Reports

Progress during 2012-13

Scientific Reports

Engineering of Nanomaterials for Biomedical Applications

Research Theme

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

Objectives

- Engineering of Bile Acid-Tamoxifen Conjugates for Breast Cancer Therapy
- Exploiting Bile Acid Amphiphiles for Colon Cancer Therapy

Principal Investigator
Avinash Bajaj

Young Investigators
Himanshu Arora
Rajender Motiani (upto April 2013)

Postdoctoral Fellows
Manish Singh
Sandhya Bansal
Ashima Singh (upto June 2013)

Research Fellows
Vedagopuram Sreekanth
Somanath Kundu
Kavita Yadav

Project Assistants
Priyanshu Bhargava

Collaborators
Sagar Sengupta, NII, New Delhi

Engineering of Bile Acid-Tamoxifen Conjugates for Breast Cancer Therapy

Cancer chemotherapy aims for specific delivery of chemotherapeutic drugs to target cancer cells leading to destruction of primary tumors in the body. Chemotherapy for cancer is associated with challenges and complications like: 1) inability of drug molecules to cross the biological barriers; 2) poor specificity leading to advent of several side effects; 3) low accumulation of drugs inside cancer cells; 4) rapid development of resistance of cancer cell to chemotherapeutic drugs. To overcome these challenges, drug delivery systems like liposomes, polymers, dendrimers, and nanoparticles have been developed for effective delivery of chemotherapeutic drugs. Clinically low outputs of existing drug delivery systems stress on the development of better drug delivery vehicles and understanding the mechanisms of these delivery vehicles.

Lipid-drug conjugates can provide alternative drug delivery vehicles to overcome the challenges and complications of existing anticancer drugs. Covalent conjugation of drugs to lipid molecules with labile linkages would help in controlled drug release from lipid-drug conjugates and would increase the efficacy of drug loading capacity. Lipid-drug conjugates provide better cellular penetration, controlled drug release, better pharmacokinetics, improved tumor accumulation, and better cellular penetration leading to enhanced therapeutic activity and lower toxicity.

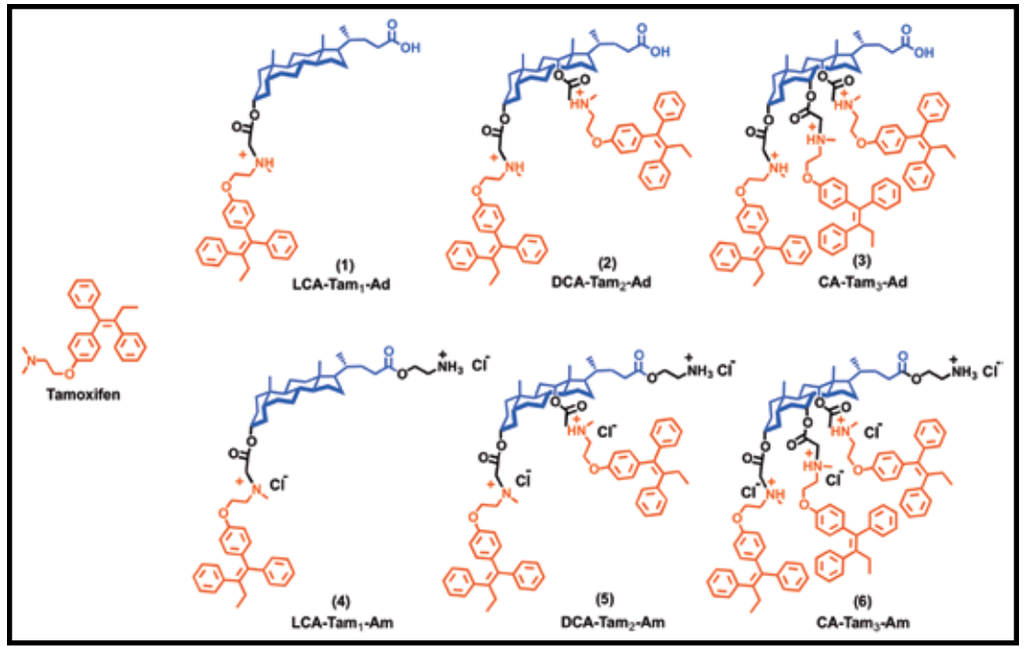
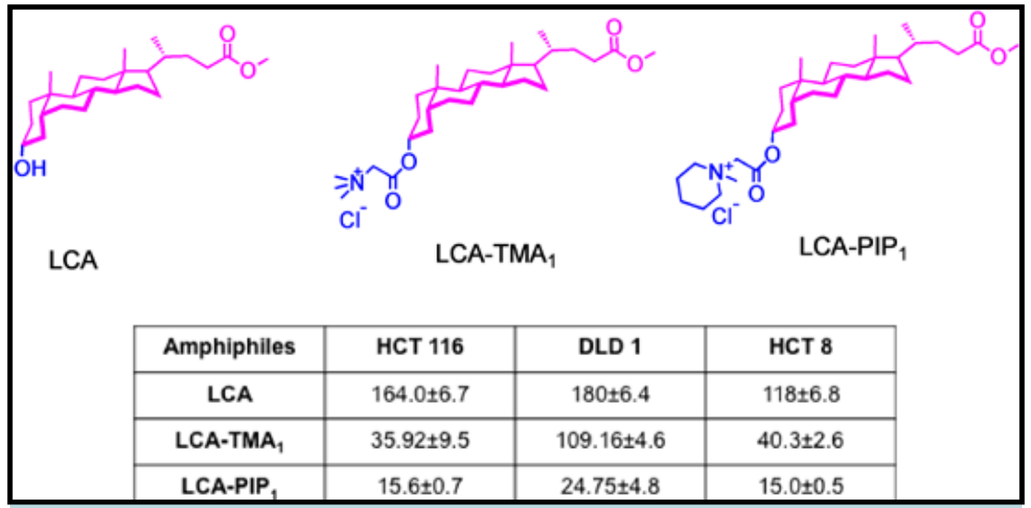


Fig. 1: Molecular structures of Tamoxifen (Tam), LCA-Tam1-Ad, DCA-Tam2-Ad, CA-Tam3-Ad, LCA-Tam1-Am, DCA-Tam2-Am, and CA-Tam3-Am used in this study.

We synthesized two series of bile acid tamoxifen conjugates using three bile acids Lithocholic Acid (**LCA**), Deoxycholic Acid (**DCA**) and Cholic Acid (**CA**) (Fig. 1). These bile acid-tamoxifen conjugates possess 1, 2, 3 tamoxifen molecules attached to hydroxyl groups of bile acids having free acid and amine functionalities at the tail region of bile acids.

The *in vitro* anticancer activities of these bile acid tamoxifen conjugates show that free amine head group based cholic acid-tamoxifen conjugate (**CA-Tam$_3$-Am**) is the most potent anticancer conjugate as compared to parent drug tamoxifen and other acid and amine head group based bile acid-tamoxifen conjugates. Cholic acid-tamoxifen conjugate (**CA-Tam$_3$-Am**) bearing three tamoxifen molecules shows enhanced anticancer activities in both estrogen receptor

Scientific Reports



Amphiphiles	HCT 116	DLD 1	HCT 8
LCA	164.0±6.7	180±6.4	118±6.8
LCA-TMA_1	35.92±9.5	109.16±4.6	40.3±2.6
LCA-PIP_1	15.6±0.7	24.75±4.8	15.0±0.5

Fig. 2: Molecular structure of bile acid amphiphiles and their IC50 values in different colon cancer cell lines.

+ve and estrogen receptor -ve breast cancer cell lines. Enhanced anticancer activity of **CA-Tam_3-Am** is due to more favorable irreversible electrostatic interactions followed by intercalation of these conjugates in hydrophobic core of membrane lipids causing increase in membrane fluidity. Annexin-FITC based FACS analysis showed that cells undergo apoptosis, and cell cycle analysis showed the arrest of cells in sub G_0 phase. ROS assays showed high amount of generation of ROS independent of ER status of the cell line indicating changes in mitochondrial membrane fluidity on uptake of the conjugate that further leads to release of cytochrome *c*, a direct and indirect regulator of ROS. The mechanistic studies for apoptosis using PCR and Western analysis showed apoptotsis by intrinsic and extrinsic pathway in ER +ve MCF-7 cells and by only intrinsic pathway in ER -ve cells. *In vivo* studies in 4T1 tumor model showed that **CA-Tam_3-Am** is more potent than tamoxifen. These studies showed that bile acids provide a new scaffold for high drug loading, and their anticancer activities strongly depend on charge and hydrophobicity of lipid-drug conjugates.

Exploiting Bile Acid Amphiphiles for Colon Cancer Therapy

Bile acids are naturally occurring steroids produced from metabolism of cholesterol in liver, and promote solubilization and absorption of fats, nutrients, and vitamins in intestine. Bile acids are facial amphiphiles in which hydrophobic and hydrophilic parts are located on opposite faces, as compared to surfactants. This unique structural aspect of bile acids contributes towards the overall amphiphilic molecular architecture leading to variety of functions including membrane interactions and cytotoxicity. For example, the hydrophobicity of bile acids is inversely related to the number of polar OH groups. The increase in hydrophobicity of bile acids confers toxicity. In humans, Cholic Acid (**CA**) and Chenodeoxycholic Acid (**CDCA**) are primary bile acids, whereas Deoxycholic Acid (**DCA**) and Lithocholic Acid (**LCA**) are secondary bile acids. Primary bile acids upon conjugation with glycine or taurine become less hydrophobic and show less toxicity, whereas secondary bile acids are more hydrophobic and thus feature more cytotoxicity. Population-based studies indicate that consumption of Western diet increases levels of fecal secondary bile acids. These excessive deposition of bile acids is responsible for oxidative DNA damage, inflammation, and enhanced cellular proliferation against colon epithelial cells leading to colon cancer.

Anticancer activities of bile acids can be attributed either to their nonspecific detergent effects or specific receptor-mediated interactions. Bile acids can trigger apoptosis by altering physical characteristics of cell membranes through non-specific interactions. Bile acids can also specifically bind to receptors leading to cellular toxicity. Different mechanisms of bile acids-induced apoptosis have been proposed including endoplasmic reticulum stress, ligand-independent activation of death receptor pathways, and mitochondrial intrinsic pathway. Both natural and synthetic bile acids show diverse bioactivities including both apoptotic and cell-proliferative behavior contingent upon the nature of their molecular architectures. To this end, numerous bile acid derivatives have been synthesized and evaluated for their anticancer activities against an array of cancer cell lines.

We hypothesized that the introduction of cationic charge to bile acids would favor the electrostatic interactions of these amphiphiles with cell membranes and therefore can improve their cytotoxic effect. Towards this goal, we synthesized four bile acid based facial cationic amphiphiles to evaluate their cytotoxicity against colon cancer cells and membrane interactions. Different cationic head group were conjugated to hydroxyl group of **LCA**, **CDCA, DCA**, and **CA** (Fig. 2). We studied the cytotoxic activities of these facial amphiphiles in two colon cancer cell lines (HCT-116, DLD-1, HCT-8) using MTT assay, and evaluated the mechanism of cytotoxicity by light microscopy and apoptosis assay. We then studied interactions of these amphiphiles with model DPPC membranes using Prodan-based hydration, DPH-based anisotropy, and differential scanning calorimetry to evaluate their differential cytotoxic

activities by varying charge, hydration, and hydrophobicity.

Activities of these amphiphiles are contingent on the nature and number of the charged head groups. Singly-charged Lithocholic Acid-based amphiphiles are most active, whereas multiple-charged amphiphiles are least active. Lithocholic Acid-based amphiphile (**LCA-PIP$_1$**) possessing Piperidine head group is ~10 times more cytotoxic with IC_{50} value 15μM as compared to Lithocholic Acid with IC_{50} of ~150 μM in HCT-116 cells. Mechanistic studies showed that **LCA-PIP$_1$** induces apoptosis in colon cancer cells through regulation of both intrinsic and extrinsic pathways. *In vivo* potential of **LCA-PIP$_1$** in tumor xenograft models, showed that a single dose of **LCA-PIP$_1$** is required to reduce the tumor burden by 75%.

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

- Vedagopuram S, Bajaj A (2013) Number of Free Hydroxyl Groups on Bile Acid Phospholipids Determines the Fluidity and Hydration of Model Membranes. *J. Phys. Chem. B* 117: 12135-12144
- Vedagopuram S, Bansal S., Motiani R, Kundu S, Muppu SK, Datta T, Panjamurthy K, Sengupta S, and Bajaj A (2013) Design, Synthesis and Mechanistic Investigations of Bile Acid-Tamoxifen Conjugates for Breast Cancer Therapy. *Bioconjugate Chem.* 24: 1468-1484.
- Sharma S, Kundu S, Reddy AM, Bajaj A, Srivastava A (2013) Design and Engineering of Stable and Biocompatible Disulfide-cross-linked Nanocomplexes of Polyamide Polyelectrolytes for Cytosolic Delivery of Entrapped Peptide Cargo. *J. Macromol. Biosci.* 13: 927-937.
- Singh M, Singh A, Kundu S, Bansal S, Bajaj A (2013) Interactions of Bile Acid based Facial Amphiphiles with Membranes Influences Their Anticancer Activities for Colon Cancer. *Biophys. Biochim. Acta Biomembr.* 1828: 1926-1937.
- Vedagopuram S, Bajaj A (2013) Fluorescence (Fluidity/hydration) and Calorimetric Studies of Interactions of Bile Acid Tamoxifen Conjugates with Model Membranes. *J. Phys. Chem. B* 117: 2123-2133.

Mechanisms of Cell Division and Cellular Dynamics

Research Theme

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. Our research group studies the molecular regulation of cellular dynamics with a view to dissect detailed biomolecular mechanisms governing dynamic cellular process. At present we are examining the molecular underpinnings of cell division and intercellular communication, two vital and highly dynamic cellular processes.

Objectives

Fidelity of mammalian cell division is ensured through tight molecular regulation, misregulation of or slippage through that leads to aberrant mitosis, chromosome missegregation and aneuploidy, which are well-established precursors to major diseases like cancer and polycystic kidney disease. We wish to uncover the molecular mechanisms of Spindle Assembly Checkpoint (SAC) inactivation by the subunits of the multi-functional molecular motor, cytoplasmic dynein. In other projects, we wish to uncover the role of the exocytic membrane trafficking machinery during cytokinesis, the physical separation of daughter cells at the end of mitosis. Our long-term interests include elucidating the mechanistic bases for biogenesis and function of novel modes of intercellular communication.

Principal Investigator
Sivaram Mylavarapu

Young Investigators
Megha Kumar
Sharmishtha Samantaray
(upto April 2013)

Research Fellows
Sagar Mahalle
Harsh Kumar
Rajaiah Pergu
Amit Sharma

The 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 earlier demonstrated that the LIC1 subunit of dynein is responsible for stripping of SAC-effector proteins from kinetochores at metaphase in a phosphoregulated manner. We are now focused on dissecting the molecular mechanism of SAC inactivation along the following lines of investigation: determine and validate the mitotic LIC1 interactome to identify potential collaborators of LIC1, and determine the significance of multi-site mitotic phosphorylation of LIC1 in its checkpoint inactivating function. In parallel, we have directed our efforts to dissect the basis for reported functional divergence between LIC1 and LIC2 during mitosis – LIC2 has been reported to function in facilitating cytokinetic progression. These studies led us to uncover an unexpected, novel function for LIC2 in regulating metaphase to anaphase progression in a SAC-dependent manner.

Our initial approach towards dissecting the reported differential mitotic functions of LIC1 and LIC2 relied on the known molecular differences between the two proteins. 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. Despite their distinct and non-overlapping functions, however, LIC1 and LIC2 share high sequence identity (~66%) and homology (~75%). Consensus secondary structure predictions **(**Fig. 1**)** 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 had postulated 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. We tested this hypothesis by attempting to functionally convert LIC1 into LIC2, by introducing regions of mutual sequence mismatch from LIC2 into LIC1. We had surmised that these protein chimeras would help to identify regions specific to both LICs that are important for their respective functions in mitosis. Accordingly, we generated several

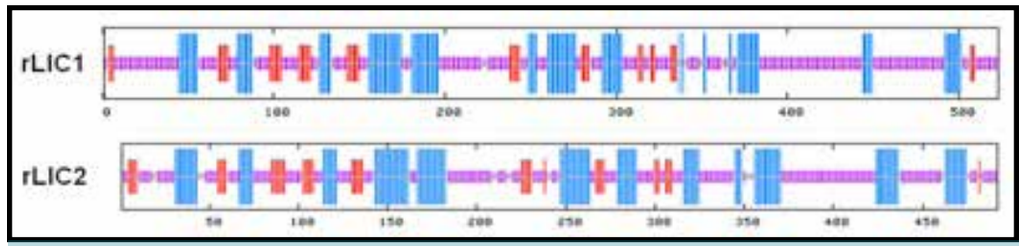


Fig. 1: Schematic of consensus secondary structure prediction for rLIC1 and rLIC2. Blue = helix, red = beta turn, purple = random coil.

Scientific Reports

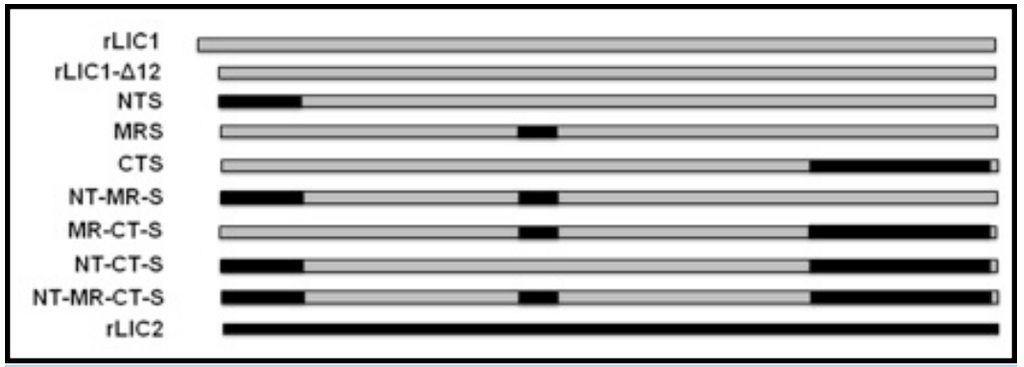


Fig. 2: Schematic of the various rLIC1-rLIC2 hybrid constructs generated.

recombinants; hybrid rLIC constructs containing rationally-selected regions of rLIC2 introduced into the rLIC1 template (Fig. 2). Care was taken not to disrupt predicted secondary structural elements.

We performed functional assays for several of these hybrids to assess for loss of LIC1 function. In short, the ability of these hybrid molecules to rescue LIC1 depletion induced metaphase arrest was assessed. However, none of the hybrid LICs showed any appreciable loss of LIC1 function. Notably, even the hybrids encompassing the C-terminal region (CTS), which constitutes the largest sequence region of difference between LIC1 and LIC2, did not show any loss of LIC1 function (Fig. 3).

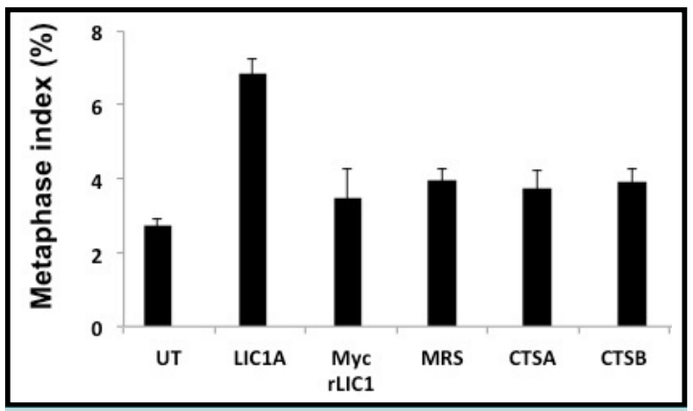


Fig. 3: The hybrid rLIC1-LIC2 constructs, MRS (mid region swap), CTSA and CTSB (C-terminal swaps) were as functional as wild type rLIC1 in complementing the loss of human LIC1 from Hela cells. All bars except UT (untransfected) are treated with siRNA against human LIC1. The labels below the last 4 bars represent the myc-tagged rLIC1 constructs used to exogenously rescue the depletion of human LIC1.

The lack of any appreciable loss of LIC1's SAC inactivation function even upon replacing its sequence significantly with non-homologous portions of LIC2 urged us to re-examine the mitotic role of LIC2 in mammalian cells. LIC2 depletion in Hela cells has previously been reported to result in a very mild cytokinetic arrest of cells; however no other mitotic phenotype is reported. Treatment of Hela cells, with the same LIC2 siRNA as reported in that paper, surprisingly showed us a potent metaphase arrest, as reported earlier for LIC1 depletion (Fig. 4). Intriguingly, the metaphase arrest phenotype was additive when both LIC1 and LIC2 siRNAs were administered to cells (Fig. 4). This observation strongly suggested that both LIC1 and LIC2 independently regulate metaphase to anaphase progression. We confirmed that the mitotic arrest due to LIC2 depletion was in metaphase and not in other stages of mitosis using immunofluoresc-ence analyses (data not shown).

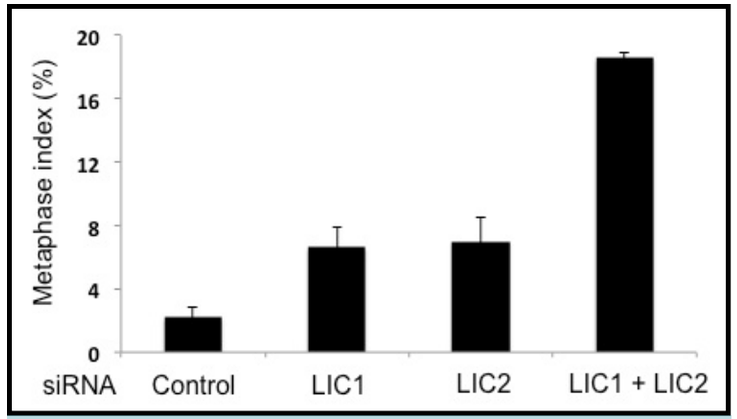


Fig. 4: Depletion of LIC2 arrests Hela cells in metaphase in a manner similar to LIC1 depletion reported earlier. The combined effect of LIC1 and LIC2 siRNA treatment is additive, suggesting independent mechanisms of action. Y-axis represents metaphase index, the fraction of total cells present in metaphase.

Cells treated with LIC2 siRNA also took significantly longer (nearly twice as long) on average to make the transition from metaphase to anaphase (Fig. 5) as compared to control cells. Live cell imaging further showed that some LIC2 siRNA treated cells spent extended times arrested in metaphase but did not progress to anaphase (Fig. 5b).

We next checked whether the LIC2 depletion mediated metaphase arrest is dependent on the presence of a functional SAC. Our experiments demonstrated that the metaphase arrest caused by LIC2 depletion is completely rescued upon co-depletion of the SAC protein Mad2 (Fig. 6). We also see the same trend upon co-depletion of another SAC protein, BubR1, with LIC2 (data not shown). This observation implicates an active SAC as the mediator of metaphase arrest upon LIC2 knockdown, in a manner similar to LIC1 depletion.

We also checked whether LIC2 depletion caused increased inter-kinetochore tension at metaphase. Physical tension between sister kinetochores at the end of metaphase leads

Scientific Reports

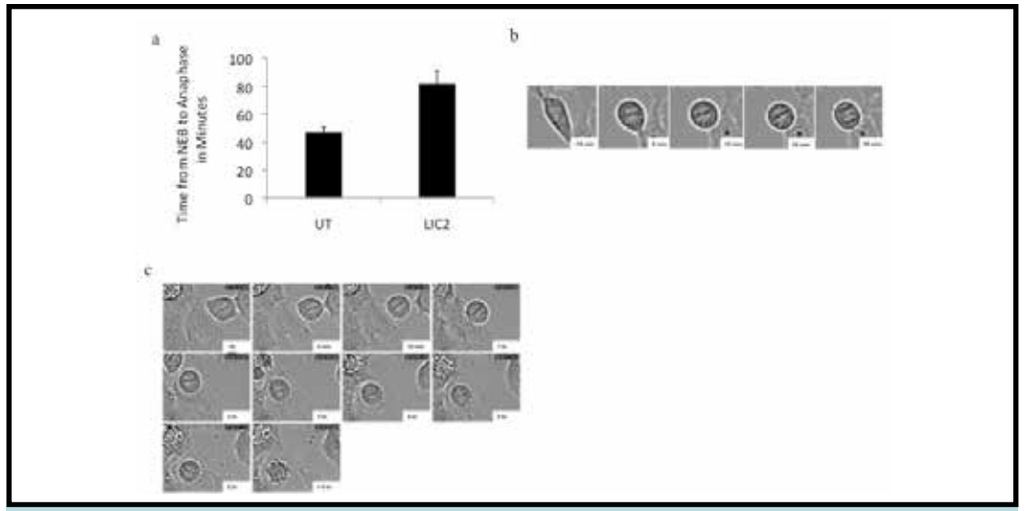


Fig. 5: Depletion of LIC2 significantly prolongs the time required for cells to transition from metaphase to anaphase. Live cell, time-lapse imaging was performed on control and LIC2 siRNA treated cells over 12 hours and the movies analyzed to determine the metaphase to anaphase timing. a) Average metaphase to anaphase timing, n = 100 cells each for control and LIC2 siRNA treated cells. NEB = Nuclear Envelope Breakdown, signifying the start of mitosis. b) Still images from a control cell showing that it completes the metaphase to anaphase transition in about 30 minutes. c) images from a LIC2 siRNA treated cell showing significantly prolonged metaphase to anaphase timing.

to enhanced inter-kinetochore stretch, which manifests as an increased average inter-kinetochore distance. LIC2 depleted metaphase cells indeed show higher average inter-kinetochore distances in metaphase cells, as compared to control cells (Fig. 7). These results suggest that LIC2 depletion leads to stalling of cells in metaphase due to inefficient inactivation of the SAC at the end of metaphase, and point to a direct role for LIC2-dynein in removal of checkpoint proteins from metaphase kinetochores.

Future Plans

Other mechanistic questions we are probing regarding the roles of LIC1 and LIC2 in mitosis are:

Does LIC2 depletion lead to the accumulation of SAC proteins on metaphase kinetochores? We will assess this by performing quantitative immunofluorescence of various SAC proteins at metaphase kinetochores in normal and LIC2 depleted cells. Accumulation of SAC proteins at metaphase kinetochores would suggest a failure of dynein (devoid of LIC2) to remove SAC proteins from metaphase kinetochores.

What are the mechanisms by which LIC1 and LIC2 phosphorylation regulate their mitotic functions? Both LIC1 and LIC2 have been demonstrated to get

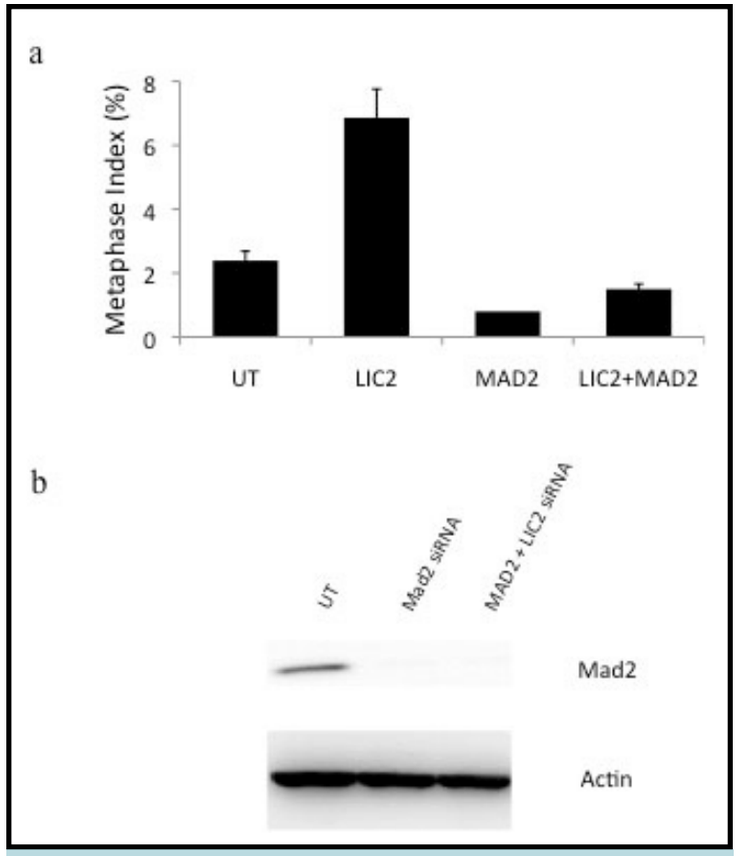


Fig. 6: LIC2 depletion-induced metaphase arrest is dependent on the presence of an active spindle assembly checkpoint. a) The metaphase arrest is abrogated completely upon co-depletion of the major spindle assembly checkpoint protein Mad2. b) Immunoblot showing specific knockdown of Mad2 upon siRNA treatment.

phosphorylated, LIC1 especially upon entry into mitosis. 4 conserved Cdk1 phosphorylation sites present in LIC1 all get phosphorylated in mitosis, and one of these is critical for LIC1 function. Interestingly, the same 4 signature sites are present in the primary sequence of the LIC2 protein. However, there is no evidence studying the phosphorylation status of these sites during mitosis. We are addressing these questions using rationally-designed phosphomutants of LIC1 and LIC2 in biochemical and functional assays.

We are also attempting to identify the mitotic interactomes of

Scientific Reports

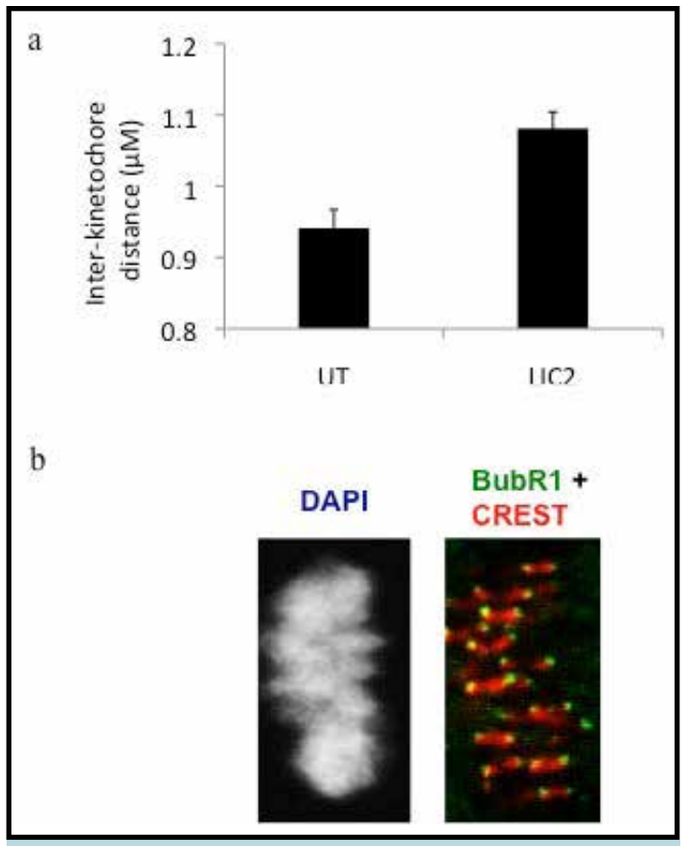


Fig. 7: LIC2 depleted metaphase kinetochores are under greater inter-kinetochore tension than in control cells. Hela cells were immunostained with antibodies against CREST (red, kinetochores), BubR1 (green) and DAPI (chromatin). Confocal images of metaphase cells were obtained and the inter-kinetochore distances of cognate sister-kinetochores were measured. At least 7 kinetochore pairs were measured per metaphase cell; the number of cells was n = 100 for control and n = 70 for LIC2 siRNA treated cells.

LIC1 and LIC2. Delineating the specific interaction partners of LIC1 and LIC2 separately would illuminate the specific mechanisms by which they function in regulating mitotic progression. LIC1 and LIC2 exist in mutually exclusive cytoplasmic dynein complexes; hence there may be a divergence of function of these two subunits with respect to mitotic progression. Alternatively, any overlaps in function and/ or biochemical interactions of these two subunits will be revealed by these approaches.

Studies on Biology of Infectious and Idiopathic Inflammation of the Gut

Research Theme

Using a model intracellular gastric pathogen, Salmonella enteric serovar Typhimurium that causes gastroenteritis, we aim to understand the molecular mechanism of infection, inflammation and general health of the gut. Salmonella induced gastroenteritis in many ways 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 such as Crohn's disease (CD) and ulcerative colitis (UC). Several cellular and molecular markers of acute inflammatory state 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 this study our main objectives will be to: (1) identify novel bacterial virulence proteins that mediate inflammatory pathways, (2) examine host molecular 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 potentially contribute to the discovery of novel biochemical pathways that may advance the development of therapeutic approaches to the treatment of intestinal inflammation.

Principal Investigator
Chittur V Srikanth

Young Investigators
Smriti Verma

Post-Doctoral Fellows
Mukesh Kumar

Research Fellows
Gayatree Mohapatra
Salman Mustafa Ahmed

Project Assistants
Swati Jain

Salmonella is able to invade the intestinal epithelial cells (IECs) through its sophisticated type three secretion system (TTSS). The interplay between the bacterial TTSS and IEC signalling is a complex molecular crosstalk, which ultimately results in massive cytoskeletal rearrangements and activation of MAPK signalling. We probed the involvement of a post-translational modification (PTM) mechanism, called SUMOylation, in *Salmonella* pathogenesis and induced inflammation. In humans the small ubiquitin like modifiers (SUMO) have three functional substrates (SUMO1, SUMO2 and SUMO3) that could be linked to hundreds of different target proteins. The SUMOylation

Scientific Reports

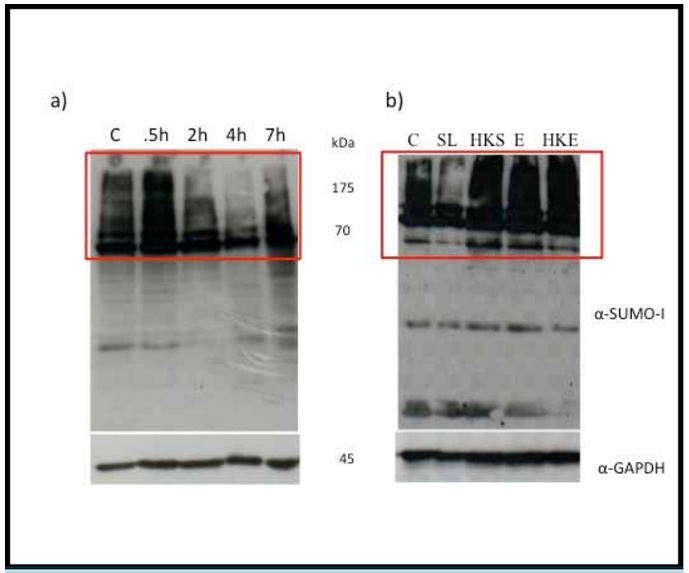


Fig. 1: Global SUMO-ylation profiling assay. A. (a) SL1344 infected HCT8 cells were lysed at different time points followed by immunoblotting for SUMO1 (top) or GAPDH (bottom). (b) HCT8 cells infected with either live SL1344 (SL), heat killed SL1344 (HKS), E. coli (E), Heat killed E. coli (HKE) or left untreated (C) followed by immunoblotting for Sumo-1 (top) or GAPDH (bottom). Area inside red coloured box represents SUMOylated proteins.

process is mediated by machinery that comprises of an E1 activating enzyme (Sae1/Sae2), an E2 conjugating enzyme (Ubc9) and several E3 ligating enzymes. 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). SUMOylation of a target protein could potentially alter several important associated aspects including its intracellular localization, interacting partner and overall function.

In our global SUMOylation profiling assay (GSPA), we infected HCT8 cells with *Salmonella typhimurium* strain SL1344. We lysed cells at different time points and immunoblotted for SUMO1 and SUMO2/3 using commercially available antibodies. Upon comparison of immunoblots of infected cells with untreated cells, we observed a significant alteration of the global SUMOylation pattern. We also checked other cell lines (such as Hela cells and T84 cells) and found similar results (data not shown). A time course GSPA revealed that the SUMOylation alteration was a dynamic process that was dependent on the time of the infection (Fig. 1a). To examine if the observed SUMO alteration was a specific response to *Salmonella* infection, we carried out GSPA using heat killed *Salmonella*, *E. coli* and heat killed *E coli*. As can be inferred from the data (Fig. 1b), the SUMO alteration specifically resulted in live *Salmonella*-infected samples and not in either *E. coli* or heat-killed *Salmonella*. These data suggest that the *Salmonella*-mediated SUMO-modification requires live bacteria and is specific for *Salmonella.*

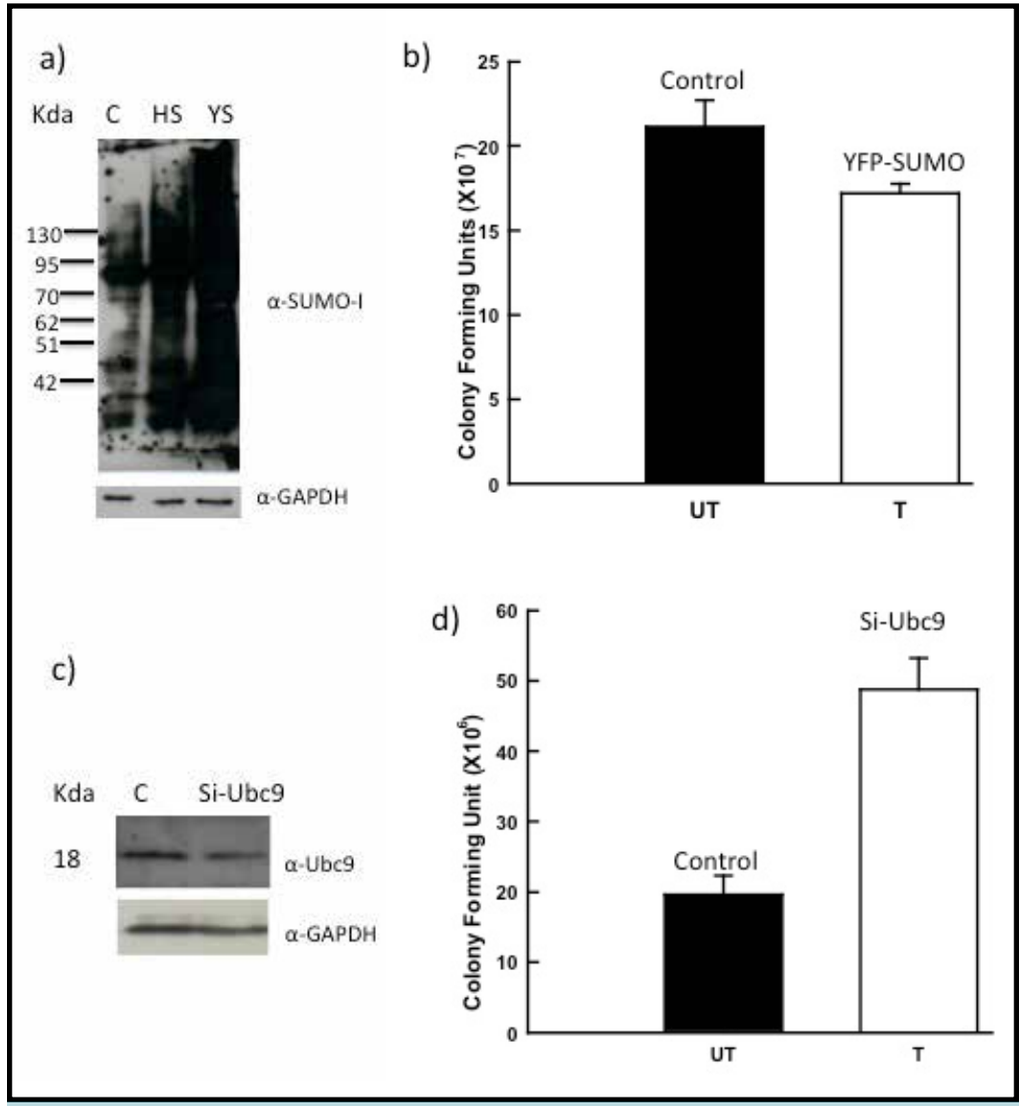


Fig. 2: Perturbation of SUMO machinery and its effect on Salmonella infection. a) Overexpression of SUMO-1 and its effects on infection: Control HCT8 cells (C) or HCT8 cells transfected with either pHis-SUMO1 or pYFP-SUMO1 followed by immunoblotting using SUMO-1(top) antibody or GAPDH (bottom). B) Control cells or YFP-SUMO1 expressing cells are infected with Salmonella for 3 hours scored for colony forming units. (Fig 2 c and D) HCT8 cells are either left untreated or transfected with SIRNA specific for Ubc9 gene followed by immunoblotting for Ubc9 (top panel C) or GAPDH (bottom). Separately the Ubc9 knocked/down cells and control untreated cells were infected with SL1344 for 3 hours and scored for colony forming units.

Scientific Reports

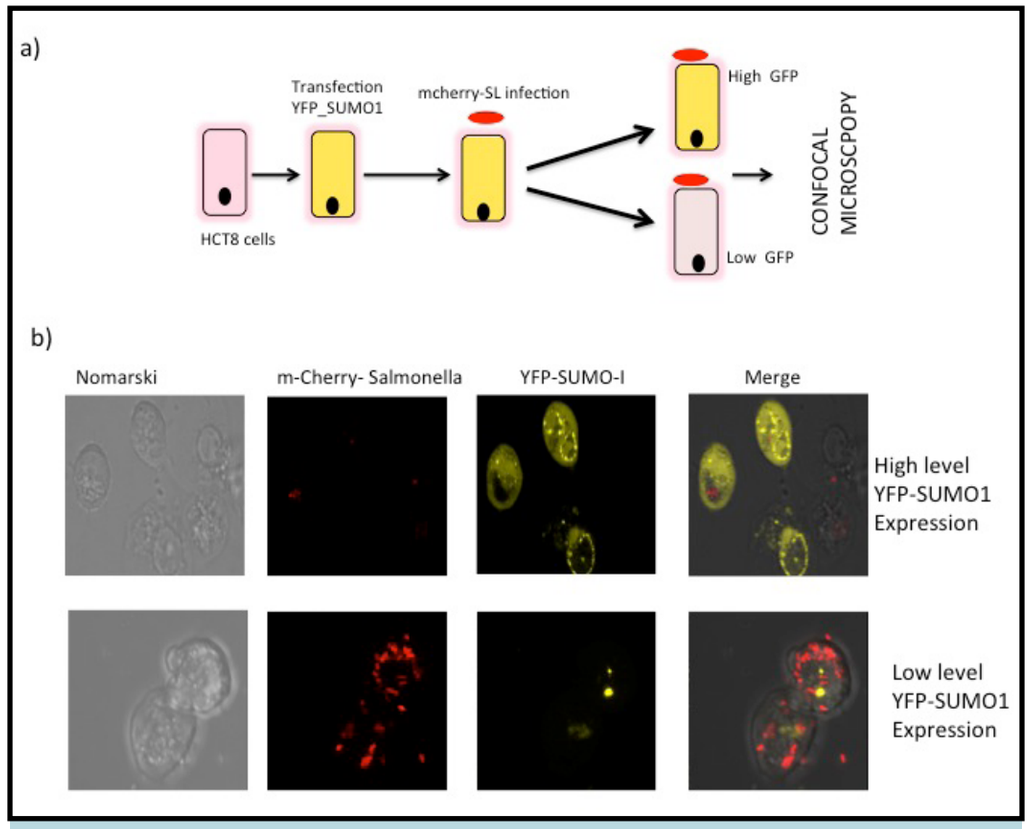


Fig. 3: SUMO overexpression inhibits Salmonella infection. (a) Schematic overview of the experiment. (b) As represented above, HCT8 cells were transfected with pYFP-SUMO1 (yellow) and infected with mCherry expressing Salmonella (red). Pictures were taken for cells expressing either high level of YFP-SUMO1 or low level of YFP-SUMO1. The extreme right panel represent merged images.

Next, experiments were carried out to ascertain the biological significance of the SUMO-alteration during infection. The host SUMO-machinery was perturbed either by (i) transient transfection of ShRNA specific for Ubc9 knock/down to down-regulate SUMO machinery, or (ii) transient transfection of plasmids pYFP-SUMO1 and pHis-SUMO1 for up-regulation of SUMO machinery (Fig. 2).

SUMO-perturbed cells (either up-regulation or down-regulation) along with untreated control were infected with *Salmonella* and the colony forming units (cfu) were scored. As can be seen in the figure 2, these experiments clearly revealed that up-regulation of SUMO machinery by overexpressing His-SUMO1 as well as YFP-SUMO1 resulted in reduced number of cfu compared to control untreated cells. The inhibition of the SUMO machinery by RNA interference on the other hand led to higher cfu compared to control cells. Thus it is evident that SUMO machinery plays a role in the intracellular survival of *Salmonella.*

To further understand the biological significance of SUMOylation during *Salmonella* infection we carried out high-resolution confocal microscopy of HCT8 cells overexpressing SUMO1 and infected them with mCherry expressing fluorescent *Salmonella*. In our analysis we deliberately focused on cells that were either expressing high levels of YFP-SUMO1 or low levels of YFP-SUMO1 (Fig. 3). Interestingly, as can be seen in figure 3, there was a significant decrease in number of *Salmonella* (red color) in cells with up-regulated SUMO machinery (yellow color). On the other hand the cells with lower YFP-SUMO1 had plenty of *Salmonella.* Thus these data reconfirm our earlier finding that SUMO machinery plays a role during Salmonella infection and is tightly regulated during infection. Any perturbation in this pathway could lead to dramatic effects on the ability of *Salmonella* to establish successful infection.

To gain deeper understanding of the mechanism of SUMO alteration during *Salmonella* infection, we scrutinized the expression levels of the unique E2 enzyme (Ubc9) of SUMO machinery. Cells infected with *Salmonella* for varying time points were lysed and immunoblotted using Ubc9 antibody (Fig. 4). We saw down-regulation of Ubc9 in infected cells, a process that started as early as 1 hr post infection. Levels of GAPDH however remained unaltered throughout the experiment (Fig. 2d). These results further validate the participation of SUMO mechanism with *Salmonella* infection.

To examine if *Salmonella*-mediated Ubc9 degradation was proteosomal machinery-dependent, we treated HCT8 cells with a proteosomal inhibitor (MG132) followed by *Salmonella* infection and immunoblotted using Ubc9 antibody. We observed that inhibition of proteasomal degradation machinery had no effect on *Salmonella*-mediated Ubc9 degradation (Fig. 4a middle panel, lane MSL). These data reveal that *Salmonella*-mediated Ubc9 degradation is independent of proteasomal machinery.

To investigate if *Salmonella* also targets other components of the SUMO machinery, we analyzed the expression of PIAS1, an E3 ligase that is known to be involved in inflammation, using real-time PCR analysis. HCT8 cells that were either left untreated or that were transfected with siRNA specific for Ubc9 (named as Ubc9KD cells) were infected and analyzed for expression of Ubc9 and PIAS1 at different time points (Fig. 4b). It was evident that the expression of both these genes was inhibited upon *Salmonella* infection, a process that began at as early as 30 minutes post infection. At 7 hours post infection however, the inhibition

Scientific Reports

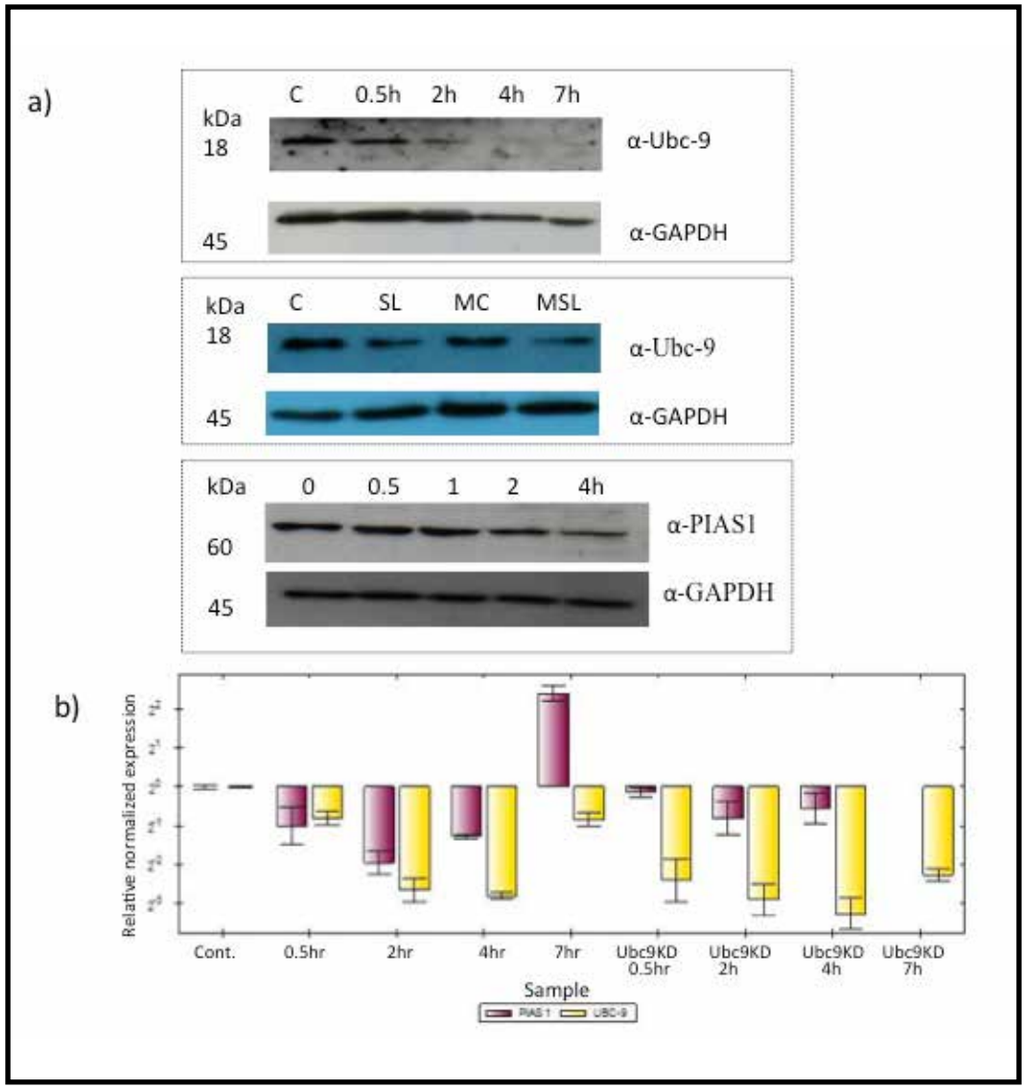


Fig. 4: Expression analysis of SUMO machinery genes. (a) SL1344 infected HCT8 cells or control untreated cells were lysed at different time points followed by immunoblotting for Ubc9 (top panel) or PIAS1 (bottom panel) GAPDH (all panels). Separately untreated HCT8 cells or those treated with proteasome inhibitor MG132 were infected with SL1344 (MSL) or left uninfected (MC) followed by immunoblotting for Ubc9 (mwiddle panel)). (b) SL1344 infected HCT8 cells or control untreated cells were lysed at different time points followed by extraction of total RNA expression analysis of 18rRNA (control), Ubc9 and PIAS1 gene were carried out. Same experiment was also carried out in SiRNA mediated Ubc9 knockdown cells (Ubc9KD). Relative normalized expression is represented here.

was much less for Ubc9 and was reversed in case of PIAS1. We found the same results in case of immunoblots of PIAS1 as shown in Fig. 4a (bottom panel). In case of the samples from Ubc9KD cells, the expression of Ubc9 remained more or less unaffected but that of PIAS1 was significantly less repressed compared to the control cells. These data suggest that *Salmonella* targets both Ubc9 and PIAS1 for modulating the host SUMO machinery. In conclusion our experiments reveal the role of SUMOylation alteration in the process of *Salmonella* infection and highlight this novel mechanism in the biology of Salmonella-host crosstalk.

Future Plans

Investigation of molecular mechanistic details of the *Salmonella*-mediated SUMO-alteration.

Examination of the consequence of SUMO-alteration in *Salmonella* replication and induced inflammation

Mass-spectrometric analysis for identification of SUMO1 and SUMO2 proteome (the reagents for these experiments have already been made ready).

Yeast 2 hybrid-based analysis the SUMO-modified proteome in infected and untreated samples.

The identified candidate proteins will be tested for their role in *Salmonella* infection as well as induced inflammation (by carrying out invasion and inflammation assays in knock/down cell line as we have earlier done (CV Srikanth *et al*, *Science* (2010) 330:390). If knock-out mouse is available for the given gene, we will examine the effect of these genes using our *in vivo* colitis model (CV Srikanth *et al*, *Science* (2010) 330:390).

The connection of the identified protein(s) for their association with the machinery of auto-immune disorders (inflammatory bowel disease such as CD and UC) in humans will also be investigated in collaboration with Dr. Vineet Ahuja (Department of Gastroenterology, AIIMS, New Delhi). A modified proposal for ethical clearance for these experiments is being prepared.

Scientific Reports

Molecular Pathophysiology of Thrombosis in Disease Conditions

Research Theme

Current research is focused on investigating hemolysis associated thrombosis in hemolytic patients. Intravascular hemolysis increases the risk of thrombosis and blood vessel blockage in different hemolytic disease conditions including thalassemia, paroxysmal nocturnal hemoglobinuria (PNH), hemolytic-uremic syndrome (HUS) and malaria. Recently we have shown that the extracellular hemoglobin (Hb)significantly regulates the activity of a plasma glycoprotein, von Willebrand factor (VWF) that serves normally the hemostatic functions (to stop bleeding) by binding to platelet and initiating clot formation at the site of injury. We hypothesize that Hb and VWF interactions play a crucial role to increase the risk of thrombosis in hemolytic patients.

Objectives

- Determining the mechanism of extracellular Hb-mediated activation and binding of VWF to platelet GP1bα that promotes thrombus formation.
- Assessing the correlation between intravascular hemolysis and elevation of prothrombotic factors such as hyper-reactive VWF in plasma of the hemolytic patients.
- Examining thrombus formation and blood vessel blockage in mice under different hemolytic conditions.

Principal Investigator
Prasenjit Guchhait

Young Investigators
Sheetal Chawla

Research Fellows
Gowtham K Annarapu
Rashi Singhal

Collaborators
Tulika Seth, AIIMS, New Delhi
Parvez Kaul, SKIMS, Srinagar
Don Mc Clain, Uni. Utah, USA

We observed that VWF-mediated platelet thrombus formation on HUVEC monolayers was increased dose-dependently in presence of extracellular HbA (adult Hb) under flow shear conditions. VWF siRNA treatment to HUVECs significantly decreased the above effects. Similarly, a significantly increasing trend in VWF-mediated platelet thrombus formation was observed in presence of HbA on immobilized sub-endothelium matrices such as collagen (type-1, type III) and fibrinogen. The effect was inhibited by anti-VWF antibody 6D1 confirming the role of Hb-VWF axis in thrombosis. We have determined the HbA binding sites on VWF. The HbA bound to A2 domain on VWF specifically at Y1605-M1606 region, which is the cleavage site for ADAMTS13 metalloprotease. A potential binding affinity was measured when Hb was perfused over VWFA2 under flow conditions using Biacore assay. As examined, HbA bound more significantly to VWFA2 when compared with other types, such as HbA_2 or HbS. On the other hand, HbA failed to bind the mutated VWFA2 (Y1605A). We also observed that HbA bound dose-dependently to A1 domain of VWF that binds to platelet GP1bα.

We are currently determining the HbA-binding site on VWFA1 using mutagenesis technology. We also have observed that HbA did not bind to VWFA3 domain. We have focused on examining interaction of HbA to A1A2A3 domain considering the crucial binding interactions of this region of VWF with platelet ligand GP1bα. Accordingly, we are searching a mechanism – how HbA interactions activate VWF to bind potentially to platelet. We also have observed that plasma VWF bound to platelet dose-dependently in presence of HbA, and the binding was significantly decreased in VWF-deficient plasma. Similarly plasma VWF also bound dose-dependently to GP1bα-expressing CHO cells in presence of HbA. Thus the above experiments suggest a crucial involvement of Hb in the regulation of VWF-GP1bα axis as well as platelet thrombus formation under hemolytic conditions.

Future Plans

To find the insights into hemolytic-associated thrombosis, we would like to determine correlation between intravascular hemolysis and elevation of prothrombotic factors in plasma of the hemolytic patients. We would also examine the thrombus formation and microvascular blockage in hemolytic mice. Hypoxia is known to induce thrombosis and hyper-coagulation. Therefore, we would like to explore the hypoxia-mediated regulation of early growth response-1 (Egr-1) and plasminogen activator inhibitor-1 (Pai-1) in promoting hyper-coagulation and thrombosis in Tibetans who are living at high-altitude (nearly 5000 m) for many generations. Similarly, we would perform the studies on hyper-coagulation, thrombosis/thromboembolism and thrombocytopenia in Dengue infections to determine the role of platelet activation, hyper-coagulation and thrombosis to the severity of thrombocytopenia in dengue hemorrhagic fever or shock syndrome.

Publications

Review/Proceedings

- Tiwari RL, Annarapu GK, Guchhait P (2013) Pathophysiological Consequences of Hemolysis in Sickle Cell Disease in “Sickle Cell Disease” (Eds. D Acuna-Costroviejo and I Rusanova). *Nova Science Publishers, Inc*. pp 69-96

Scientific Reports

Structural Biology of Bacterial Surface Proteins

Research Theme

Understanding architecture and biogenesis of bacterial surface proteins and assemblies, and their role in adhesion, host interaction and biofilm formation is the theme of our research. We are currently focusing on hair-like surface organelles known as pili from Gram-positive pathogenic and beneficial bacteria and trying to understand their strategies in pathogenesis and probiosis.

Objectives

Several pathogenic bacteria assemble pili on their cell surface to adhere to the host cell and promote pathogenesis. Pili are made up of protein subunits called pilins, which are connected to one another by either non-covalent interactions (in Gram-negative bacteria) or covalent bonds (in Gram-positive bacteria). Substantial structural information already exists for pili from Gram-negative pathogens and it is beginning to emerge for pili from Gram-positive pathogens. More recently, the pili have also been identified in beneficial Gram-positive bacterial strains (e.g. probiotics). Knowledge of the mechanisms by which bacterial pili adhere to host cells is crucial in biomedicine for controlling the adhesion of bacterial pathogens and probiotics. Our objective is to investigate pilus architecture and assembly mechanism in beneficial and pathogenic bacteria, and understand their strategies in pili-mediated host-bacterial interactions in health and disease.

Principal Investigator
Vengadesan Krishnan

Young Investigators
Susheela Kushwaha

Research Fellows
Deepak Singh
Priyanka Chaurasia

We have started our studies with two bacterial strains; *Lactobacillus rhamnosus* GG (gut flora) and *Streptococcus oralis* (oral flora) as gut and oral cavity are the most densely colonized sites of bacteria in the human body.

A. Pili from *Lactobacillus rhamnosus* GG

Lactobacillus rhamnosus GG, a widely used probiotic strain, is a Gram-positive bacterium. It is clinically well studied for its perceived health benefits, which are often manifested as the stabilization of microbial imbalances in the human gut, antagonism of pathogenic bacteria, and modulation of protective innate immunity. Two types of pilus gene clusters (*SpaCBA* and *SpaFED*) have been identified in the *L. rhamnosus* GG genome. The *SpaCBA* pilus cluster is comprised of genes for a major pilin *SpaA*, two minor pilins SpaB and SpaC, and a pilin-specific sortase. Similarly, the *SpaFED* pilus cluster consists of genes for a major pilin SpaD, two minor pilins SpaE and SpaF, and a pilin-specific sortase. Based on immunogold electron microscopic studies, and in comparison with pili from Gram-positive pathogens, the position and function of each individual pilin subunit in the assembled pilus fiber of the *L. rhamnosus* GG has been predicted. The repeating SpaA comprise the backbone. The "cell wall-anchoring" SpaB and "adhesive" SpaC are found at the base and tip of the pilus fiber. Interestingly, in contrast to the pili from Gram-positive pathogens, the SpaC (and, to a lesser extent, SpaB) is distributed sporadically throughout the shaft of the SpaCBA pilus. It is thought that this distribution could enhance adherence to the intestinal mucosa and epithelial layer, and thus increase the relative longevity and transient colonization of *L. rhamnosus* GG cells in the gut. The SpaFED pili are not observed in the laboratory conditions despite the presence of the *SpaFED* gene in the genome. It is possible that the SpaFED pili are produced in intestinal tract, similar to the Tad pilus in *Bifidobacterium brevei* that was expressed only *in vivo* in mouse intestine.

In order to better understand the structural features of the SpaCBA and SpaFED pilus, and the mechanisms of assembly and adhesion, we continued the study of the individual pilin constituents. The initial recombinant clones for the pilins of *L. rhamnosus* GG were provided by Dr. Airi Palva group, University of Helsinki, Finland.

i) SpaA

The crystallization condition that produced flower like crystals earlier was further optimized. The optimized condition containing 0.35 M trisodium citrate and 14% PEG 3350 yielded single large crystals suitable for X-ray diffraction (**Fig.1**). Initial X-ray diffraction data from SpaA crystals were collected up to a resolution of 2.6 Å at home source and to 2.0 Å on the BM14 beamline at the ESRF (Grenoble, France). SpaA crystals belong to the monoclinic space group *C*2, with unit-cell parameters a = 227.9 Å, b = 63.2 Å, c = 104.3 Å, β = 95.1°. Calculation of the Matthews coefficient (V_M = 2.44 Å^3 Da^{-1}) suggests the presence of five molecules in the asymmetric unit, which corresponds to a solvent content of 50%. Since SpaA has a limited sequence identity with known structures, our initial attempts to obtain phases by molecular replacement were not successful. Moreover, as methionine or cysteine residues are not present in SpaA, phase calculation by selenium or sulfur single-wavelength anomalous dispersion (SAD) was not feasible. Hence, attempts to solve the phasing problem through the use of heavy-atom derivatization and other possible ways are under progress.

Scientific Reports

Fig. 1 : Crystals of SpaA

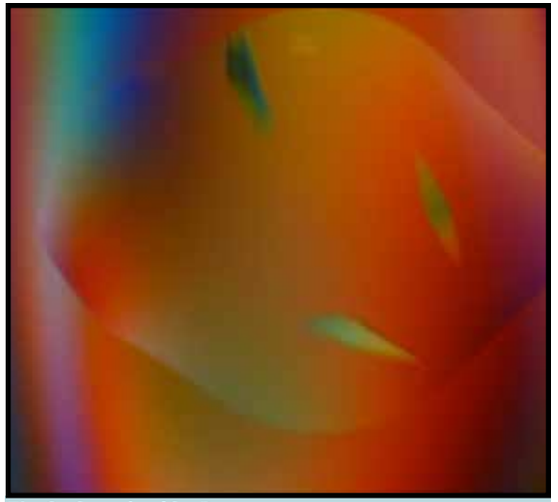


Fig. 2 : Crystals of SpaD

ii) SpaD

The SpaD was purified using affinity and gel-filtration chromatographic techniques. Attempts to crystallize SpaD by screening various conditions using in-house liquid handling robotic system (Mosquito) as well as manual crystallization set up produced small crystals. Optimization of one of the conditions (0.2M disodium phosphate and 20% PEG3350) yielded single crystals (Fig. 2) suitable for diffraction. However the diffraction data were not indexable likely due to severe twinning associated with crystal packing. Attempts to get indexable diffraction data by producing crystals in different conditions and limited proteolysis are in progress.

B. Pili from *Streptococcus oralis*

Two different pilus types (PI-1 and PI-2) have been identified in mitis group oral streptococci bacteria from the genomic analysis. The PI-2 shows certain unique characteristics while the PI-1 resemble a typical three-pilin architecture (one major and two minor pilins). PI-2 is comprised of only two pilins. The PI-2 pilus is present as a single structure on the bacterial surface, in contrast to the typical multitude of pilus structures per bacterial cell. In addition to sortases, the PI-2 also encodes a signal peptidase like protein (SipA), which is required for pilus assembly. However, SipA does not contain the two conserved residues, serine and lysine, required for typical signal peptidase activity. The mitis group streptococci adhere to tooth surfaces and epithelial tissues for colonization and biofilm formation, and compete against their wash out by salivary flow to the digestive tract. The mitis group streptococci (*S. oralis, S.mitis, S. sanguinis*) appear on the tooth surfaces and epithelial tissues within few minutes after cleaning. They are directly associated with gingivitis and caries. Also they are the primary colonizers providing adhesion sites for other bacteria to form oral biofilm called plaque.

In order to understand unique structural features of PI-2 pilus and their role in adherence, plaque formation, and their similarities and differences with PI-1 plus of other commensals and pathogens, we have initiated our parallel study on PI-2 from *S. oralis*. Cloning of pilus subunits (PitA and PitB), sortases (SrtG1 and SrtG2), and SipA are under progress.

Future Plans

Structural and functional investigations of pilin subunits and sortases from *L. rhamnosus* GG will be continued. While phasing by traditional heavy atom derivatization continues, a mutational study has also been planned to replace few residues to methionine in SpaA to examine the possibility of Selenium-SAD phasing. Lysine methylation and limited proteolysis will be carried out to improve diffraction quality of SpaD crystals.

Expression, purification, crystallization will be attempted for pilus subunits (PitA and PitB), sortases (SrtG1 and SrtG2), and SipA from *S. oralis* after completion of recombinant cloning.

Publications

Original peer-reviewed articles

- Deepak S, Ingemar von O, Airi P and Vengadesan K (2013) Purification, Crystallization and Preliminary Crystallographic Analysis of the SpaA Backbone-pilin Subunit from Probiotic *Lactobacillus rhamnosus* GG *Acta Cryst. (2013). F69, 1182-1185*
- Vengadesan K, Macon K, Sugumoto S, Mizunoe Y, Iwase T and Narayana SVL (2013) Purification, Crystallization and Preliminary X-ray Diffraction Analysis of the *Staphylococcus epidermidis* Extracellular Serine Protease Esp *Acta Cryst F* 69:49-52.
- Krishnan V, Dwivedi P, Kim BJ, Samal A, Macon K, Ma X, Mishra A, Doran KS, Ton-That H and Narayana SV (2013) Crystal Structure of *Streptococcus agalactiae* tip pilin GBS104; A Model GBS Pili Assembly and Host Interactions *Acta Cryst D* 69:1073-1089.

Scientific Reports

Deciphering Signaling by Immune Regulators during Effector-triggered Immunity

(New Proposal)

Research Theme

A specific layer of plant defense, termed effector-triggered immunity (ETI), rely on sensing of a pathogen specific effector (termed avirulence factor; Avr) by a cognate plant resistance (R) protein. My research focus on deciphering the molecular signaling routes that initiate and characterize ETI.

Objectives

- Understanding molecular assemblies and trigger of ETI
- Deciphering the role of inositol-derivatives in ETI signaling

Principal Investigator
Saikat Bhattacharjee

Unlike most conventional stepwise cascades of receivers and transducer proteins, the robust and rapid ETI response is likely mediated by direct functions of defense regulators. It has been already demonstrated that specialized assemblies of immune players, maintained at distinct organellar locales, are targets of specific injected effectors of the bacterial pathogen *Pseudomonas syringae* pathovar tomato (DC3000). My objective is to understand how these molecular complexes of R and R-associated proteins (resistasome complex) are maintained on membranes and how effector-induced perturbations function and relay the signal to the nucleus to cause massive transcriptional reprogramming (Fig. 1).

Primarily, the focus is on inositol-derived compounds that have multi-faceted role in protein assemblies on membranes and as secondary messengers in most eukaryotic systems. It is planned to investigate the involvement of inositol-phosphates (InsPs) in plant mutants altered in defense responses to DC3000. The main outcome is to identify whether specific inositol compounds mediate actions of ETI responses. In a parallel approach attempts would be initiated in deciphering the steady-state

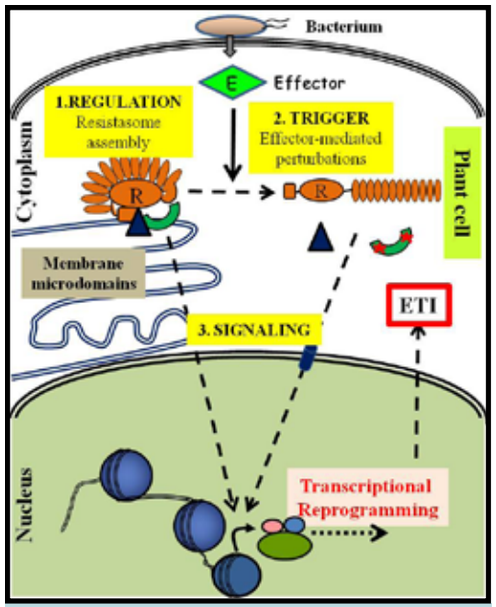


Fig. 1 : Schematic representation of key steps of ETI initiation

protein-protein interactions platforms of resistance proteins and immune regulators on lipid interfaces in the plant cell and how pathogen effectors modulate signaling. The main goal of this approach is to characterize how associations of immune players with resistance proteins regulates and triggers immunity. Lastly, inositol phosphates have been previously reported to co-crystallize with auxin and jasmonic acid hormonal components. Utilizing this information, it is aimed to elucidate the synergistic and antagonistic cross-talk between hormonal pathways and how pathogen effectors or induced ETI impinge of this network.

Future Plans

Several genetic combinations of plant mutants of key immune players with genes involved in inositol biosynthesis and modifications have generated that display altered ETI responses. Preliminary studies indicate that either misregulation of a negative immune regulator or deficiencies in specific inositol phosphates are some features that contribute to the altered immunity in these mutants. Further characterization of these lines have the potential to identify components that signal downstream of important immune players. It is widely accepted that resistance proteins do not function in a vacuum. I have identified putative candidates that associate with specific pools of resistance proteins and likely function to modulate activities. These candidates contain phosphatidylinositol (PtdIns) binding/modifying motifs and may mediate tight regulation of immunity under native state and induction, only upon perception, of an invading pathogen. In vitro binding and in vivo pull-down assays of these proteins with PtdIns are in the pipeline. Appropriate marker transgenic plants are being obtained/generated that will be useful in following up PtdIns kinetics during ETI. In summary, these studies may unravel how a plant deploys intelligent defenses.

Scientific Reports

Complement Regulatory Proteins in Autoimmunity

(New Proposal)

Research Theme

Regulators of the complement cascade like complement factor H (CFH) and the CFH-related proteins (CFHR) enable accumulation of activated complement components on pathogenic surface while preventing complement activation on healthy host cell surfaces. CFH and CFHR function antagonistically to modulate appropriate complement activation. CFHR deficiency in some cases leads to autoimmunity whereas in many other cases remain asymptomatic. Development of autoantibody clearly indicates a breach in B cell tolerance and one major immune component that can participate in breaching B cell tolerance and disruption of B cell negative selection are dendritic cells. Study focusing on understanding the differences in activation and differentiation status of DC and B cells from symptomatic and asymptomatic individuals with CFHR deficiency will help in better understanding of the innate and adaptive immune interface and development of autoimmunity.

Objectives

- Understanding the role of complement regulatory proteins in maintenance of B cell tolerance.
- Understanding the tolerogenic role of DC in B cell negative selection.

Principal Investigator
Priyadarshini Chatterjee

Future Plans

Availability of sample: The material for the proposed study would be blood samples from adult and children. Blood samples can be collected from hospitals around the city and from pediatric centers. Buffy coats from blood samples would be used to isolate peripheral blood mononuclear cells (PBMC). PBMC would be used for assays and different cell populations will be identified by the following markers: plasmacytoid dendritic cells: $CD11c^{-}CD123^{+}CD303^{+}HLA\text{-}DR^{+}$, myeloid DC: $lin^{-}CD11c^{+}CD1c^{+}$, immature B cells $CD19^{+}IgD^{+}CD27^{-}CD10^{+}CD38^{hi}CD24^{hi}$ and mature naïve B cells: $CD19^{+}IgD^{+}IgG^{-}CD27^{-}CD10^{-}CD38^{lo}CD24^{lo}$. Wherever needed, these populations can be isolated using multi-color flow-cytometry based sorting.

Apoptotic cell assays: CFHR proteins with known similarity in binding specificity to CFH can possibly regulate the amount of CFH deposited on apoptotic cells, thereby helping to fine-tune proper apoptotic clearance. Aberrant apoptotic cell clearance can induce a cytokine milieu that is pro-inflammatory which is most often associated with a rise in autoimmunity. CFH associated with apoptotic cells (AC) in patient blood samples can be assessed by separating these cells using biotinylated-Annexin V over magnetic columns and then the isolated cells can be stained for bound CFH using anti-CFH antibody. AC derived from CFHR-deficient and sufficient individuals will be used to stimulate DC and downstream activation will be measured.

Assay to measure DC activation: CFH and complement C3 degradation products deposited on antigen presenting cells like DC can be actively taken up and presented to cognate T cell. Anti-CFH, anti-C3b and anti-C3d antibodies will help assess the amount of CFH and C3 degradation products deposited on DC. Binding of these products to complement receptors like CR1 and CR3 is known to activate downstream signaling cascade with upregulation of MHCII, co-stimulatory molecules like CD80 and CD86 and the release of proinflammatory cytokines like IL-12, IL-10, IFNα and immune modulators like B cell activating factor (BAFF). mRNA levels for cytokines will be assessed by real-time PCR and the intracellular protein message for the same will be measured using flow-cytometry. Levels of activation molecules and activated signaling intermediates can be measured by flow-cytometry using specific antibodies. Homozygous or heterozygous deletion at the CFHR1/3 loci can result in quantitatively different CFH and C3 products deposited on DC thereby differentially activating these cells. Tolerogenic versus chronically-activated DC would release qualitatively and quantitatively different immune modulators. Many such immune modulators, like BAFF have established roles in B cell negative selection and establishing tolerance.

Assay to elucidate mechanism of activation of anti-CFH B cells: Proportion of anti-CFH B cell clones in immature and mature B cell compartments will be studied. B cells stimulated polyclonally will be measured for their anti-CFH producing ability by ELISA and ELISPOT assays. Activation status of B cells will be analyzed by measuring MHCII, CD80, CD86, CD69 and HSA expression. Sensitivity of B cells to immune-modulators released by DC like BAFF will be estimated by analyzing levels of activated downstream signaling intermediates like Pim2. Similar experiments can also be performed for other DC-derived factors and their corresponding signaling intermediates in B cells. This could help

establish DC-B cell interface as an active component responsible for the break in B cell tolerance in CFHR-deficient homozygotes. Apoptotic debris coated with CFH can provide a two-signal stimulation for anti-CFH B cell clones, firstly, through the BCR and secondly, through ligation of endocytic TLR7 and TLR9 with nucleic acid components of internalized apoptotic cells. In an animal model of lupus it has been demonstrated that dual engagement of BCR and TLR can strongly induce autoantibody production from self-reactive B cells. To assess if TLR signals are essential in activating the anti-CFH B cell clones association of signaling intermediates like MyD88 and TRIF with endosomal compartment will be studied by microscopy. Levels of p-Syk, p-PLCγ2 or the p-MAPKs in anti-CFH B cells would also determine the activation status of these autoreactive B cell clones. Activation of B cells by BCR and TLR engagement does not require T-cell help. Antibody isotypes like IgG3 are known to be predominant in T-cell independent B cell activation and therefore measurement of serum titers of anti-CFH IgG isotypes could further emphasize the mode of auto-reactive B cell activation.

Long term plan: One limitation in the human system is that the observations made in the phenotype and functionality of immune cells cannot be correlated to the singular deficiency of CFHR proteins since immunological dysfunction and pathogenicity are often cumulative effects of multiple genetic deficiencies and polymorphisms. Also, access to lymphoid tissue in humans has practical limitations. Therefore, generation of mice with deletion in the *CFHR1/3* loci may be required in due course for a more detailed understanding of the mechanistic role of CFHR in DC activation and breakdown of B cell tolerance.

Investigating Molecular Mechanism in the Ubiquitin Mediated Signalling in Cellular Pathways

Research Theme

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 processes.

Objectives

Human genome analysis and proteomics 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 different types of ubiquitin chains. It has been revealed that the dysregulation of deubiquitinating enzymes leads to diseases like Parkinson, Alzheimer, Ataxia, heart disease and different types of cancer. Our aims are to understand the possible molecular mechanisms underlying these diseases.

Principal Investigator
Tushar Kanti Maiti

Young Investigators
Sushmita Bhattacharya

Research Fellows
Pranita Hanpude
Roshan Kumar

BRCA1-associated protein-1 (BAP1), a member of ubiquitin-C-terminal hydrolases (UCH) family of deubiquitinating enzymes, was discovered through its interaction with the RING finger domain of tumor suppressor protein BRCA1. BRCA1 forms a heterodimer with BRCA1 associated RING domain 1 (BARD1) protein and shows ubiquitin ligase (E3) activity. BAP1 interferes with BRCA1 ligase function. Unlike other ubiquitin C terminal hydrolases, BAP1 contains UCH domain and nuclear localization signal domain. It has been demonstrated that both deubiquitination and nuclear localization are required for BAP1-mediated tumor suppressor function. BAP1

Scientific Reports

Proteins	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m x 10^5 ($M^{-1}S^{-1}$)	K_d (ITC) (μM)	K_d(SPR) (μM)
UCHL1	0.03	0.029	8.87	0.07	-
UCHL3	0.02	5.9	2773	0.01	-
UCHL5 (1-240)	10.72	8.7	8.1	-	12.0
BAP1 (1-240)	2.98	2.1	7.1	4.0	-

Table1: Enzymatic activity and ubiquitin binding for all UCH members

interacts with transcription regulator Host Cell factor 1 (HCF1) without affecting its deubiquitinating activity. The C-terminal of BAP1 also interacts directly with a transcription factor YY1. The association of BAP1 with HCF1 and YY1 implies its role in cell cycle progression. BAP1 is located on chromosome 3p21 in a region that shows loss or deletions in numerous cancers including lung cancer, breast cancer, uveal melanoma and mesothelioma. Recent genetic studies in BAP1 show that missense, deletion mutations are observed throughout the BAP1 gene and the high propensity of mutations are observed in catalytic domains in uveal melanomas, mesotheliomas, and melanocytic tumors and lung cancers. Mutations like A95D and G178V in lung cancer were shown to abolish the deubiquitinating activity of BAP1. Considering all aspects, it is pertinent to say that BAP1 has diverse physiological roles in controlling cellular function.

In the present report we are aiming to elucidate the possible mechanism underlying the cancer due to the mutations of BAP1. We have sub cloned full length BAP1 gene into pET 28a vector. However, the expression of BAP1 is very less and hence we have segregated this gene domain-wise. As the propensity of the cancer-associated mutations in BAP1 is observed in the UCH domain, we sub cloned UCH domain into pGEX-6P1 vector, overexpressed in *E. coli* and purified according to GST purification protocol. UCH domain yielded a significant amount of protein and initial attempt has been made to crystallize. The UCH domain protein did not produce diffraction quality crystals. We have measured the enzymatic behavior and ubiquitin recognition using Ub-AMC hydrolysis assay, surface plasmon resonance and isothermal titration calorimetry. These results suggested catalytic domain of BAP1 is very similar to catalytic domain of UCHL5, a proteasome associated DUBs of same family (Table 1).

We are aiming to understand the molecular basis of inactivation of A95D and G178 V mutants. Sequence alignment and homology modeling with UCHL5 structure showed that the 95 position is one turn below in the same helix with catalytic residue of cysteine. One can argue that the mutation of Asp leads to the disruption of rigid catalytic network

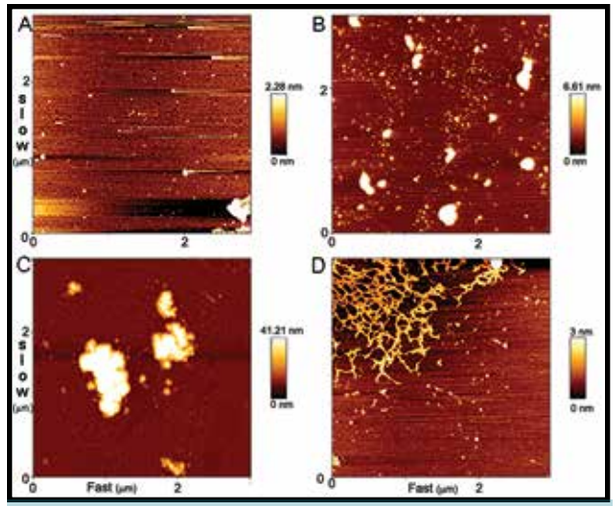


Fig. 1: AFM image of WT and A95D mutant BAP1 (1-240). 10μl of 10μM sample is placed in freshly cleaved mica surface and air-dried. The salt and buffer are removed by washing with water. AFM image is taken in non-contact mode (tapping mode). (A) BAP1 WT (1-240) at 250C, (B) BAP1 WT (1-240) at 370C incubation for 10h, (C) BAP1 A95D (1-240) at 250C, (D) BAP1 A95D (1-240) at 370C incubation for 10h.

and subsequently the reason for catalytic inactivation. To validate this hypothesis we have mutated the alanine at 95 position with different amino acids based on size charge and polarity. We have replaced Ala with Gly, Leu, Ile, Val, Ser, Cys, Thr, Glu, Gln, His, Arg and Phe and studied their enzymatic property and thermal stability. Enzymatic measurement shows that only Gly shows similar activity where as Ser, Cys, Thr and Val show substantially reduced activity. Other amino acids do not show any catalytic activity. The size *vs.* activity correlation plot suggested that size of amino acid side chain plays a major role in deciding the enzymatic property of proteins. Incorporation of large size chain in the rigid catalytic cleft might cause structural destabilization. The thermal melting curves for all mutants are consistent with enzymatic activity data. The cancer associated Asp mutant shows very unusual type of thermal melting curve. All mutants show the Tm value ranging from 38-49 ^oC with sharp transition where as A95D mutant shows Tm value ~ 65^0C with shallow transition. Circular dichroism data showed also presence of secondary structural elements even after heating the protein solution to 75 ^oC. This data clearly suggest formation of protein aggregate.

To validate the aggregation, we have performed Atomic Force Spectroscopy (AFM) measurement of wild type and A95D proteins after 10h incubation at 37^0C (Fig. 1). The AFM results showed that the A95D mutant formed a protein fibril upon incubation on 37 ^oC where as wild type protein does not produce any protein aggregate. This finding leads to the conclusion that mutation of Alanine at 95 position leads to the structural destabilization causing protein aggregation and consequently abolishing its catalytic activity.

Overexpression of full-length protein BAP1 and A95D mutant BAP1 was performed in HEK293T and HepG2 cancer cell lines to corroborate the

mutation effect and cellular aggregation of BAP1. Expression of wild type BAP1 is observed both in cytosol and in the nucleus. Two C-terminal NLS signaling domains of BAP1 facilitate the nuclear localization, which plays a major role Histone 2B deubiquitination. Due to fibrillar aggregation A95D mutant BAP1 does not penetrate to the nucleus, consequently perinuclear and cytosolic accumulation are observed.

Future Plans

Systems biology approach to understand the cancer-causing mutations observed in different cancers will be the attractive strategy for molecular analysis of cancer. iTRAQ label based differential mass spectrometric-based proteomic approach will be adopted to elucidate the molecular mechanism. We will overexpress different BAP1 mutants and wild type protein in BAP1 knockout cell line NCI H226 and HEK 297T cell line (no endogenous expression). Proteomic and subsequent pathway analysis will provide a clue for possible mechanism BAP1's involvement in cancer.

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

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

Intrinsic Signals that Regulate Skeletal Muscle Structure and Function

Research Theme

We are studying cellular differentiation and its regulation using the skeletal muscle, a tissue essential for mobility, posture, support and body temperature maintenance. In order to achieve our goals, we study skeletal muscle development, differentiation, homeostasis, regeneration and their regulation using in vivo and in vitro approaches.

Objectives

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

- Study the role of a family of skeletal muscle specific genes called skeletal muscle Myosin heavy chains (MyHCs), critical to skeletal and heart muscle structure and function, in skeletal muscle development, differentiation, regeneration and homeostasis, using specific MyHC conditional targeted mouse alleles in vivo and myogenic C2C12 cells in vitro.
- Investigate the mechanisms underlying the temporal and spatial regulation of expression of specific MyHCs, during development and differentiation in vivo and in vitro and how specific signalling pathways (e.g. Wnt-β-Catenin pathway) regulate MyHC expression and muscle differentiation.
- Identify genes with dynamic expression characteristics during C2C12 myogenic differentiation, initially using a candidate gene approach to be expanded into a genome wide screen, and investigate their functional requirement in myogenesis, in vitro and in vivo.

Principal Investigator
Sam J. Mathew

Young Investigators
Masum Saini

Research Fellows
Samta Marwaha

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

Scientific Reports

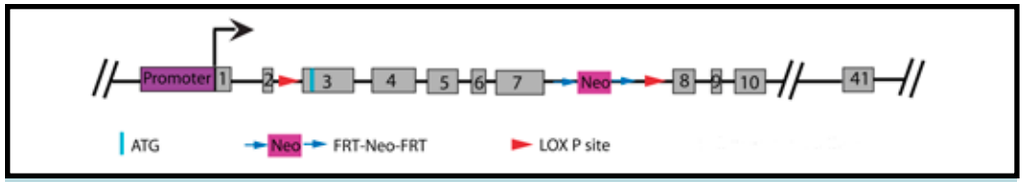


Fig. 1: Schematic showing targeting strategy used to generate MyHC-embfl/+ animals. The MyHC-embryonic genomic region depicting the promoter, exons (numbered grey boxes), introns (black lines connecting exons), start codon in exon 3 (blue line), LoxP sites flanking exons 3 ad 7 (red triangles), and the FRT-Neo-FRT cassette(blue triangles with box showing Neo).

In order to investigate the role of skeletal muscle specific MyHCs during development and differentiation, we successfully generated a conditional targeted allele for MyHC-embryonic (*MyHC-emb*$^{fl/+}$) in mouse, the first MyHC

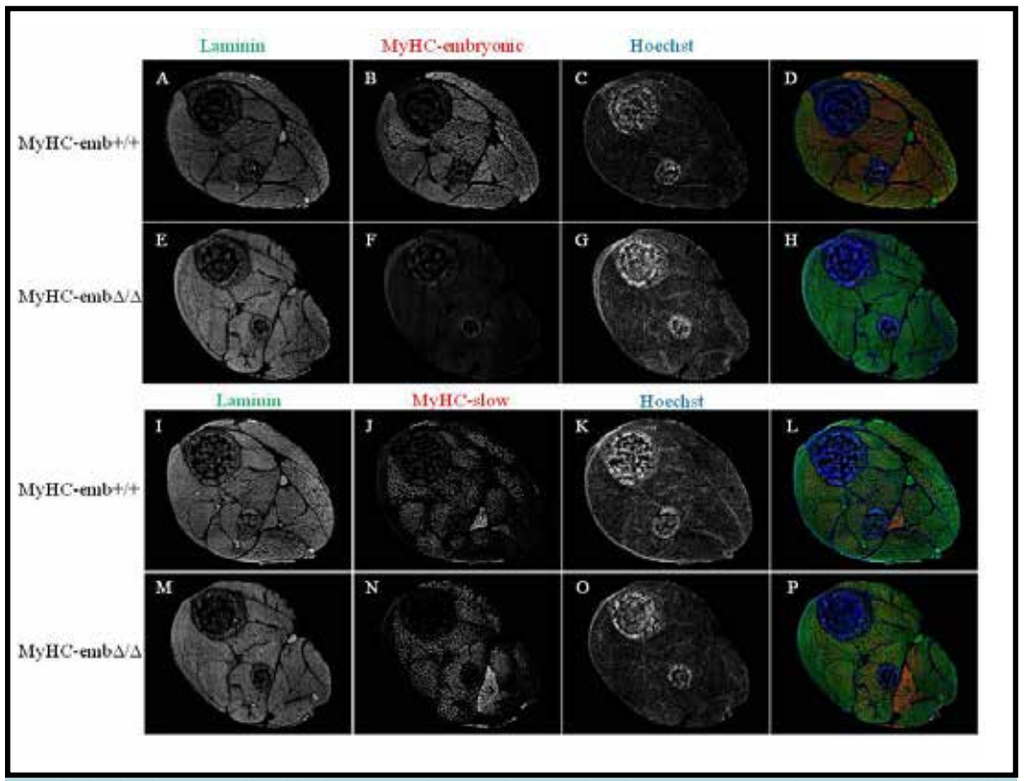


Fig. 2: Confocal images of hind limb sections showing that MyHC-embryonic protein is absent in MyHC-embΔ/Δ muscle, compared to MyHC-emb+/+ muscle, by immunofluorescence. Wildtype (A-D) and MyHC-embΔ/Δ (E-H) P0 shank cross-sections immunolabeled for Laminin for basement membrane (A, E), MyHC-embryonic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

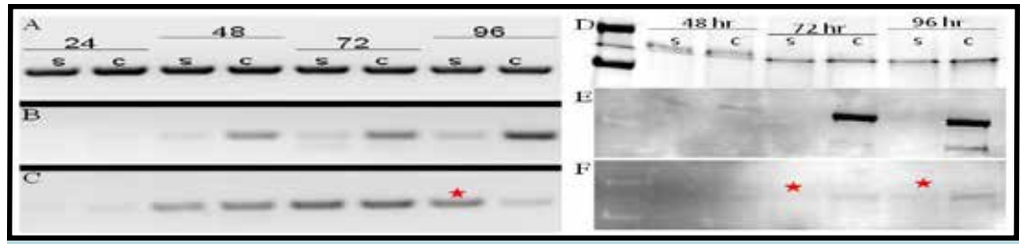


Fig. 3: Semi-quantitative RT-PCR (A-C) and western blot (D-F) on MyHC-embryonic knockdown and control cells. A-C: Semi-quantitative RT-PCR on control (c) and MyHC-embryonic siRNA knockdown (s) C2C12 cell cDNA samples from 24, 48, 72 and 96 hours post-transfection, for GAPDH control (A), MyHC-embryonic (B) and Myogenin (C). Myogenin levels have decreased by 96 hours post differentiation in control but remains high in MyHC-embryonic knockdown cells (red-asterisk in C). D-F: Western blot on control (c) and MyHC-embryonic siRNA knockdown (s) C2C12 protein lysates from 48, 72 and 96 hours post-transfection, for Laminin as loading control (D), MyHC-embryonic (E) and MyHC-slow (F). MyHC-embryonic levels are significantly low, verifying the knockdown efficiency while MyHC-slow levels are lower at 72 and 96 hour time points (red asterisks in F), in the MyHC-embryonic knockdown lysate compared to control.

to be expressed during development, by flanking exons 3 to 7 with LoxP sites (Fig. 1). Using this allele, a knockout for MyHC-embryonic was generated (*MyHC-emb*$^{\Delta/+}$), by crossing floxed mice (*MyHC-emb*$^{fl/fl}$) to *HPRT*$^{Cre/+}$ mice. Mice homozygous for the knockout allele (*MyHC-emb*$^{\Delta/\Delta}$) were generated by crossing heterozygous parents and we found that the frequency of homozygous mutant progeny was less than half that of the expected Mendelian ratio, suggesting that the *MyHC-emb*$^{\Delta/\Delta}$ genotype is semi-lethal in utero. We also verified that the *MyHC-emb*$^{\Delta/\Delta}$ allele is null for MyHC-embryonic, by immunofluorescence, using an antibody specific for MyHC-embryonic (Fig. 2). In order to determine the role of MyHC-embryonic in myogenic differentiation *in vitro*, we also studied the effect of siRNA mediated MyHC-embryonic knockdown on C2C12 cell myogenic differentiation. We found that MyHC-embryonic specific siRNA treatment reduced MyHC-embryonic transcript and protein levels significantly (Fig. 3).

Further, MyHC-embryonic specific siRNA treatment resulted in elevated levels of Myogenin at late differentiation stages (Myogenin is one of four muscle regulatory factors or MRFs, crucial to muscle differentiation), caused a reduction in the levels of MyHC-slow, compared to control cells (Figure 3), and decreased the number of reserve cells which are the myoblast cells that differentiate to produce myotubes and myofibers (Fig. 4).

We also found that Wnt-β-Catenin signalling from the myogenic lineage is crucial in regulating MyHC-embryonic expression. In *Pax7*$^{iCre/+}$*; Porcn*$^{fl/Y}$ animals, where Wnt secretion from the myogenic

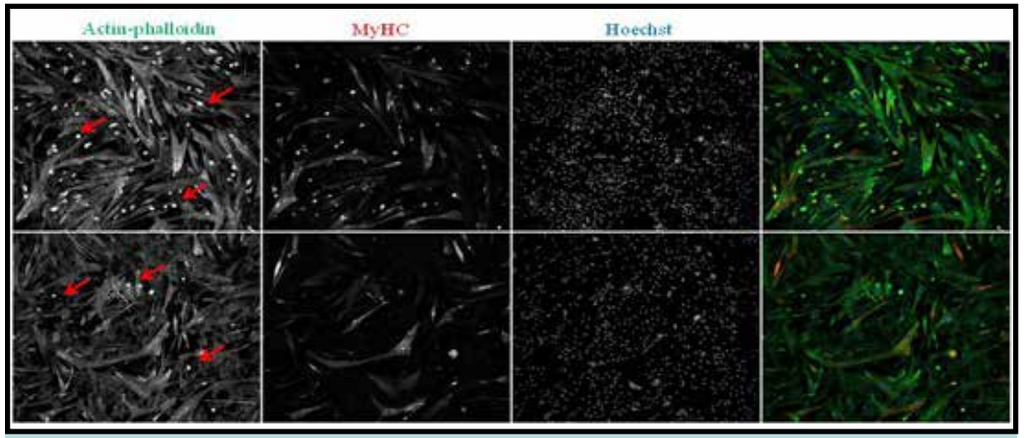


Fig. 4: siRNA mediated MyHC-embryonic knockdown in C2C12 cells during myogenic differentiation causes defective myogenesis. C2C12 cells labeled with Phalloidin for F-actin, MyHC for differentiated myofibers and hoechst for nuclei. siRNA mediated knockdown of MyHC-embryonic (bottom panel) causes improper differentiation compared to control cells (upper panel); MyHC-embryonic knockdown wells have fewer mono-nuclear reserve cells which fuse to produce myofibers (red arrows), compared to control wells and fewer differentiated myofibers.

lineage cannot occur due to the lack of Porcupine (porcn) function, which prevents Wnt processing, MyHC-embryonic protein level was upregulated in whole muscle lysates, compared to control muscle lysates (Fig. 5). This suggests that Wnt-signals originating from the myogenic lineage are important in regulating MyHC-embryonic levels, through an unidentified mechanism. It is possible that Wnt-signals regulate myogenic regulatory factors (MRFs), which are known to be crucial in regulating MyHC levels and muscle differentiation. This will be tested by generating *in vitro* reporter constructs for the MyHC-embryonic promoter and transfecting them into C2C12 cells. The role of Wnt signals in skeletal muscle differentiation *in vitro* will also be tested using recombinant Wnt protein treatment of C2C12 cells and evaluating differentiation.

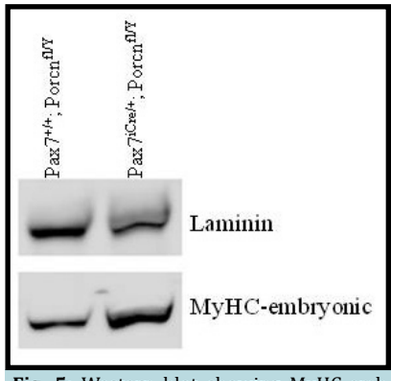


Fig. 5: Western blot showing MyHC-emb up-regulation in the absence of Wnt signals from the myogenic lineage. Western blot using P0 muscle protein lysates from Pax7iCre/+; Porcnfl/Y (right) compared to Pax7+/+; Porcnfl/Y (left) indicate that MyHC-embryonic protein levels are upregulated when Porcn is deleted in the myogenic lineage; laminin is the loading control.

Future Plans

We will start functional characterization of the *MyHC-emb*$^{\Delta/\Delta}$ to investigate whether lack of MyHC-embryonic leads to aberrant developmental or regenerative myogenic differentiation. We will also initiate genetic crosses to generate animals with the correct genotype to perform conditional deletion of MyHC-embryonic during development, regeneration and homeostasis.

We will continue our analysis of the effect of siRNA-mediated MyHC-embryonic knockdown on *in vitro* myogenesis, to understand the function of MyHC-embryonic during differentiation. We will also expand these studies to include additional MyHCs, MyHC-perinatal and -slow, which are also developmental MyHCs similar to MyHC-embryonic.

We also aim to understand the role of Wnt- β-Catenin signaling in regulating MyHC-embryonic expression during *in vitro* myogenesis, using C2C12 cells. We will identify enhancer regions of MyHC-embryonic that respond to Wnt-signals and also extend these studies to include all of the developmental MyHCs, to obtain a clear understanding of how Wnt-β-Catenin signals regulate MyHCs and differentiation. These studies can later be validated *in vivo*, using transgenic mice.

In an attempt to identify new genes critical to myogenic differentiation, we will employ a candidate gene approach, focusing on studying the expression dynamics of specific transcriptional co-repressors during C21C2 myogenic differentiation by semi-quantitative reverse transcriptase PCR (RT-PCR). From studies on *Drosophila* and other models, it is clear that multiple co-repressors are involved in myogenesis, especially by regulating muscle regulatory factor (MRF) activity. We will systematically study the expression dynamics of co-repressors and identify those that potentially have a role in regulating differentiation, which will then be functionally analyzed during *in vitro* myogenesis.

Scientific Reports

Structural Biology of Regulatory Events in Physiological Processes

Research Theme

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

Objectives

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

Principal Investigator
Dinakar M Salunke

Young Investigators
Jasmita Gill
Alka Dwevedi

Research Fellows
Abha Jain
Anamika Singh
Harmeet Kaur
Sarita Chandan Sharma
Ashish Kumar, NII, New Delhi
Sharad Vashisth, NII, New Delhi

Collaborators
Akhilesh K Tyagi,
NIPGR, New Delhi

The hallmark of acquired immune system is the remarkable specificity in its recognition repertoire that not only counters the invading pathogen but also ensures self-non self discrimination. Although immune system has evolved to discriminate finer differences between the molecules, degeneracy in immune response has often been observed. In order to understand the issue of specificity/degeneracy against an invading pathogen we began work upon a common immune evading virus known as Influenza A Virus. Therefore it would be interesting to investigate the issues of specificity/degeneracy against neutralizing epitopes on influenza virus hemagglutinin.

The influenza hemagglutinin's antigenic regions have previously been mapped. Hemagglutinin3 (near the receptor binding region) has an unusual protruding loop that has been previously shown to have neutralizing effect. Therefore a seven amino acid (WTGVTQN) epitope from that loop region was selected for further studies. All the hemagglutinin sequences from influenza research database (flu database) were extracted and aligned, of which 9 maximally varying analogues were synthesized and purified. Epitope was conjugated to 4 carrier protein and of which 3 conjugates were used to study physiological response in terms of degeneracy against the analogues. Polyclonal humoral response has shown differential degenerate response against different analogues and also overall differential degenerate response in the case of the three different conjugates. When the booster was given with lowest responding peptide conjugate we have seen an increase in the overall cross-reactivity as well as against the lowest responding peptide. The degenerate profile was remained to be the same when the peptide-conjugate was injected in 4 different strains of mice (Balbc, Black6, fvbj and carter). Two monoclonal antibodies 1A5 (KWTGVTQN-TT) and 4B1 (KWTGVTQN-KLH) were generated. Hybridoma cells were injected in the peritoneal cavity of mice to generate antibody in ascites. Both the antibodies were then purified. Fab was generated by papain digestion and purified and analysis of differential antigen recognition is in progress.

The structural proteomics of allergenic seed proteome of eggplant *(Solanum melongena),* was explored. A 45 kDa protein, SM80.1 showing weak homology with other 7S vicilins for which preliminary crystallographic studies were presented earlier, was further refined building almost entire model of the protein. The model was refined at 1.5 Å to an Rfree of 0.22 and Rwork of 0.21. The overall crystal structure of SM80.1 indicates that it is a homotrimer consisting of 393 residues in each monomer of which only residues 274-293 are structurally disordered. A monomer subunit is composed of two similar domains further subdivided into a core and a loop sub domain. 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. This is the first native structure in this family of proteins with only one disordered region. Each domain of SM80.1 forms a central cavity to facilitate ligand binding. It was found that N terminal β-barrel domain has a pyroglutamate molecule whereas an acetate molecule was present in the C-terminal domain. Superimposition of two domains shows that both the ligands exist exactly at the same position. These ligands may have a probable role in structural integrity and formation of swiss-roll topology. This protein might be acting as a depository of pyroglutamate and acetate, which are required in different metabolic pathways or could be a source of carbon and nitrogen in the germination process. Along with above Mg is also present in the structure. Surface electrostatic potential map of the protein showed an uneven distribution of charge on the protein surface. Although there was a little difference, the basic structure was close to those of 7S Adzuki bean, canavalin, AraH1 and phaseolin. All

these structures have very low sequence identity, suggesting that multiple and varied sequences can yield similar three-dimensional structure and explaining evolutionary divergence.

Another protein, SM80.2, was also purified from the defatted seed powder by 80% ammonium sulphate fractionation. The purified protein corresponds to a molecular weight of 11.7 kDa as analyzed by mass determination using mass spectrometry. N-terminal sequencing was also done for the purified protein which identified 20 residues of the polypeptide. The purified protein shows homology with other known 2S albumin family proteins. The protein was crystallized to obtain hexagonal shaped crystals which belonged to space group P6, with unit-cell parameters a = b = 87.48, c = 49.67 Å. As no homologous crystal structures of closely-related proteins were available, *ab initio* phasing was pursued utilizing eight inherited sulphur atoms (cysteine). Experimental anomalous intensities were used by the *PHENIX* package to obtain initial phase information. Data were collected on single crystal, first to 1.87 Å, which were used for high resolution structure refinement, and then to 2.5 Å, at synchrotron source (BM14, ESRF, Grenoble) which were used for SAD phasing. Program SOLVE successfully identified in total seventeen sulphur sites from the Bijvoet pair differences. A preliminary model was built automatically by *Autosol.* A total of 150 resides were built and 8 chains were placed with R_{work} and R_{free} values of 38.32 and 41.81 respectively.

The seeds of a medicinally important plant, *Mucuna pruriens*, well known for its pharmacological properties such as anti-Parkinson, antineoplasty, antioxidant, antidiabetic and antivenom activity. A 21 kDa protein, MP-4, was earlier purified and almost fully sequenced through N-terminal sequencing of proteolytic digests The protein showed weak homology with kunitz type trypsin inhibitor from *Delonix regia*. Relatively poor serine protease inhibitory activities were confirmed when tested against trypsin and chymotrypsin to which MP-4 was found to bind at K_D values of 2.65e-6 and 3.39e-6 respectively. A low model was earlier refined at 3.0A resolution of the protein crystals that belonged to space group $P2_1$ with unit-cell parameters a = 45.2 Å, b=50.9 Å, c = 69.4 Å and β=104.2^0. Molecular replacement refinement of the partial model was stagnated at 3.0 Å resolution due to poor data quality. We have now re-crystallized MP-4 and collected excellent data at high resolution in space group $P2_12_12$, with unit-cell parameters a = 51.0 Å, b=69.2 Å and c = 45.3 Å. Using the previously refined partial model, structure is now built completely and refined starting from 3.4 Å resolution, gradually increasing resolution to avoid the bias in the model. The current refinement statistics are Rcryst 27.3 % and Rfree 28.8 % at 2.8 Å resolution.

Expansins form a large multigene family found in rice, wheat and other cereal genomes that are involved in the expansion of cell walls as a tissue grows have been associated with pollen allergy. The present work emphasizes structural studies on rice pollen allergenicity caused due to EXPB present abundantly in pollen. The beta-expansin proteins: EXPB1; EXPB5; EXPB9; EXPB10 are being explored towards structural analyses. High level expression and purification of EXPB9 was reported. The protein has further been characterized biochemically/biophysically and subjected to extensive crystallization attempts. Similarly, EXPB 10, another closely related protein was also expressed and purified. The total protein yield in case of EXPB9 was 12.0 mg and that of EXPB10 was 10.4 mg from 10 L of culture. Refolding confirmation, presence of disulfide linkages, homogeneity and oligomeric states of EXPB9 and EXPB10 was done using mass spectrometry and other biophysical techniques such as analytical gel permeation, dynamic light scattering, spectrometry, spectropolarimetry and infra-red spectroscopy. Crystallization attempts on these proteins are being pursued.

Future Plans

Structural proteomics of plant seed allergens and pollen allergens from *Oryza sativa* will be continued towards crystallographic analysis and structure-function correlation. 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

- Tapryal S, Gaur V, Kaur KJ, Salunke DM. (2013) Structural Evaluation of a Mimicry-recognizing Paratope: Plasticity in Antigen-antibody Interactions Manifests in Molecular Mimicry. *J. Immunol*. 191:456-463.
- Bhowmick A and Salunke DM. (2013) Limited Conformational Flexibility in the Paratope may be Responsible for Degenerate Specificity of HIV Epitope Recognition. *Int. Immunol.* 25:77-90.
- Sharma R, Lomash S and Salunke DM. (2013) Putative Bioactive Motif of Tritrpticin Tevealed by an Antibody with Biological Receptor like Properties. *PloS One* (in Press)

Reviews/Proceedings

- Salunke D M, Kaur H and Gill J (2012) New paradigms in antibody specificity: Structural Biology of Antigen Recognition by Germline Antibodies in 'Biomolecular Forms and Functions' (Ed. M. Bansal and N. Srinivasan) World Scientific Publishing, pp. 173

New Faculty Members

Saikat Bhattacharjee

Postdoc
University of Missouri-Columbia, USA
Boyce Thomson Institute for Plant Research, USA

PhD 2004
Purdue University, USA

Research Interests

Plant defenses against invading pathogens are bi-layered in nature. The second layer of immunity, referred to as effector-triggered immunity, is deployed in plants that involves sensing specific secreted effectors (termed avirulence factors; Avr) through cognate resistance proteins (R). While new resistance specificities are constantly being identified, molecular signaling events that define ETI remain unknown. Signal transductions in ETI defies 'stepwise, linear arrangement of transducers and receivers' and is likely is triggered as a result of effector-activities that alter steady-state dynamics of key immune players. Robustness and rapidity of ETI signaling has been suggested to be mediated by intricate and interlinked sectors that further relay signals to protein networks called hubs. Inositols, considered as 'the cellular language of eukaryotic signal transduction', likely mediate and/or are involved in multiple aspects of immune signaling. Inositol derivatives, direct several key cellular processes such as mRNA export, apoptosis, plant hormone signaling and control of transcription. Inositol-modified lipids (phosphatidylinositols, PtdIns) determine architecture of most eukaryotic membranes. Inositol phosphates (InsPs) function as key secondary messengers. The broad goal of my lab is to elucidate inositol-dependent influences on ETI. To achieve this understanding, the following objectives will be pursued:

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

Selected Publications

- Gassmann, W., and Bhattacharjee, S. (2012). Effector-triggered immunity signaling: From gene-for-gene pathways to protein-protein interaction networks. Mol. Plant Microbe Interact. 25: 862-868.
- Bhattacharjee, S., Halane, M.K., Kim, S.H., and Gassmann, W. (2011). Pathogen effectors target Arabidopsis EDS1 and alter its interactions with immune regulators. Science 334: 1405-1408.

Priyadarshini Chatterjee

Postdoc
Pediatric Biology Center, THSTI, Gurgaon, India
Children's Hospital, Harvard Medical School, Boston, USA

PhD 2009
National Institute of Immunology, New Delhi, India

Research Interests

The complement system is a powerful part of the host innate immune defense and is aimed to damage and eliminate microbes and modified self-cells. Regulation of complement activation is therefore of extreme importance in order to enable accumulation of activated complement components only on pathogenic surface while preventing complement activation on healthy host cell surfaces leading to autoimmunity. Examples of regulators of the complement cascade are complement factor H (CFH) and the CFH-related proteins (CFHR) that have structural homology to CFH and function as CFH antagonists. CFHR deficiency in some cases leads to autoimmunity whereas in many other cases remain asymptomatic. Development of autoantibody clearly indicates a breach in B cell tolerance and one major immune component that can participate in breaching B cell tolerance and disruption of B cell negative selection are dendritic cells. Study focusing on understanding the differences in activation and differentiation status of DC and B cells from symptomatic and asymptomatic individuals with CFHR deficiency will help in better understanding of the innate and adaptive immune interface and development of autoimmunity.

The following specific objectives are proposed to be carried out:

- Understanding the role of complement regulatory proteins in maintenance of B cell tolerance.
- Understanding the tolerogenic role of DC in B cell negative selection.

Selected Publications

- Balthasar A. Heesters, Priyadarshini Chatterjee*, Young-A Kim, Santiago F. Gonzalez, Michael P. Kuligowski, Tomas Kirchhausen and Michael C. Carroll (2013) Endocytosis and recycling of immune complexes by follicular dendritic cells enhances B cell binding and activation. *Immunity*. 38:1164-75.
- Priyadarshini Chatterjee, Ritesh Kumar Tiwari, Satyajit Rath, Vineeta Bal, Anna George. (2012) Modulation of antigen presentation and B cell receptor signaling in B cells of Beige mice J. Immunology. 188:2695-702.
- Gautam N Shenoy*, Priyadarshini Chatterjee*, Sheetal Kaw, Snigdha Mukherjee, Deepak K Rathore, Vineeta Bal, Satyajit Rath, Anna George. (2012) Recruitment of memory B cells to lymph modes remote from the site of immunization requires an inflammatory stimulus. J. Immunology. 189:521-8

** equal contribution*

Achievements and Academic Activities

Publications

Original peer-reviewed articles

1. Sharma R, Lomash S and Salunke DM. (2013) Putative Bioactive Motif of Tritrpticin Tevealed by an Antibody with Biological Receptor like Properties. *PloS One* (In Press)
2. Vedagopuram S, Bajaj A (2013) Number of Free Hydroxyl Groups on Bile Acid Phospholipids Determines the Fluidity and Hydration of Model Membranes. *J. Phys. Chem. B* 117: 12135-12144
3. Vedagopuram S, Bansal S., Motiani R, Kundu S, Muppu SK, Datta T, Panjamurthy K, Sengupta S, and Bajaj A (2013) Design, Synthesis and Mechanistic Investigations of Bile Acid-Tamoxifen Conjugates for Breast Cancer Therapy. *Bioconjugate Chem* 224: 1468-1484.
4. Deepak S, Ingemar von O, Airi P and Vengadesan K (2013) Purification, Crystallization and Preliminary Crystallographic Analysis of the SpaA Backbone-pilin Subunit from Probiotic *Lactobacillus rhamnosus* GG. *Acta Cryst F* 69: 1182-1185
5. Sharma S, Kundu S, Reddy AM, Bajaj A, Srivastava A (2013) Design and Engineering of Stable and Biocompatible Disulfide-cross-linked Nanocomplexes of Polyamide Polyelectrolytes for Cytosolic Delivery of Entrapped Peptide Cargo. *J. Macromol. Biosci.* 13: 927-937.
6. Singh M, Singh A, Kundu S, Bansal S,Bajaj A (2013) Interactions of Bile Acid based Facial Amphiphiles with Membranes Influences Their Anticancer Activities for Colon Cancer. *Biophys. Biochim. Acta Biomembr.* 1828: 1926-1937.
7. Vedagopuram S, Bajaj A (2013) Fluorescence (Fluidity/hydration) and Calorimetric Studies of Interactions of Bile Acid Tamoxifen Conjugates with Model Membranes. *J. Phys. Chem. B* 117: 2123-2133.
8. Vengadesan K, Macon K, Sugumoto S, Mizunoe Y, Iwase T and Narayana SVL (2013) Purification, Crystallization and Preliminary X-ray Diffraction Analysis of the *Staphylococcus epidermidis* Extracellular Serine Protease Esp. *Acta Cryst F* 69:49-52.
9. Krishnan V, Dwivedi P, Kim BJ, Samal A, Macon K, Ma X, Mishra A, Doran KS, Ton-That H and Narayana SV (2013) Crystal Structure of *Streptococcus agalactiae* tip pilin GBS104; A Model GBS Pili Assembly and Host Interactions. *Acta Cryst D* 69:1073-1089.
10. Tapryal S, Gaur V, Kaur KJ, Salunke DM. (2013) Structural Evaluation of a Mimicry-recognizing Paratope: Plasticity in Antigen-antibody Interactions Manifests in Molecular Mimicry. *J. Immunol*. 191:456-463.
11. Bhowmick A and Salunke DM (2013) Limited Conformational Flexibility in the Paratope may be Responsible for Degenerate Specificity of HIV Epitope Recognition. *Int. Immunol.*25:77-90.

Reviews/Proceedings

12. Salunke D M, Kaur H and Gill J (2012) New paradigms in antibody specificity: Structural biology of antigen recognition by germline antibodies in '*Biomolecular forms and functions*' (Ed. M. Bansal and N. Srinivasan) World Scientific Publishing, pp. 173.
13. Rajiv Tiwari, Gowtham Kumar Annarapu, Prasenjit Guchhait. 2013. Pathophysiological consequences of hemolysis in sickle cell disease in "*Sickle Cell Disease*" (Ed. D Acuna-Costroviejo and I Rusanova). Nova Science Publishers, Inc. pp 69-96

Lectures delivered / Conferences attended/ Visits abroad

Dr. Dinakar M. Salunke

- Delivered an invited lecture on 'New paradigms in antibody specificity: Structural biology of antigen recognition by germline antibodies' at International Conference on Biomolecular Forms and Functions: A Celebration of 50 years of the Ramachandran Map, Indian Institute of Science, Bangalore, 8-10 January 2013.
- Delivered an invited lecture entitled 'Dynamics and diversity of signaling the antigenic encounter: New paradigms in antigen-antibody recognition' at Viswa-Bharati, Santiniketan, West Bengal, 19 February, 2013.
- Delivered a keynote address entitled 'Structural biology of antigen recognition' at Venkateswara College, New Delhi 14 March, 2013.
- Delivered an invited lecture entitled 'Molecular replacement approach for X-ray crystal structural determination: Theory & practice' at RRCAT, Indore 10 April 2013.
- Delivered an invited lecture entitled 'New paradigms in antibody specificity: Structural biology of antigen recognition' at SYSCON 2013, AIIMS, New Delhi, 23 August 2013.
- Participated in G8 Science Summit: 7-9 March 2013, INSA, New Delhi.
- Participated in Second Summit of South Asian Science Academies: 24-27 September, 2013, INSA, New Delhi.
- Participated in review session of the ESRF CRG beamline BM14 at ESRF, Grenoble, France. 6-7 November, 2012.
- Participated in the inaugural meeting of Scientific Advisory Board - International Institute for Biotechnology, Nigeria at University of Nigeria, Nsukka, Nigeria, 5-8 February, 2013.
- Participated in 7th General Assembly and Conference of Global Network of Science Academies, Brazil. 24-27 February 2013.
- Participated in 19th Council of Scientific Advisors meeting of International Centre for Genetic Engineering and Biotechnology at Trieste, Italy. 5-8 May, 2013.

Dr. Sivaram V. S. Mylavarapu

- Participated in fifth Young Investigators' Meeting (YIM) held at Jodhpur from February 9 - 13, 2013.

Dr. Avinash Bajaj

- Participated in the fifth Young Investigators' Meeting (YIM) held at Jodhpur from February 9 - 13, 2013.

Dr. Sam J. Mathew

- Presented a lecture "Drosophila as a model organism" in the workshop on "Eukaryotic Model Organisms" at the Regional Centre for Biotechnology (RCB), Gurgaon, October 12-16, 2012.

Dr. C. V. Srikanth

- Attended India Alliance DBT-Wellcome Trust Fellows meeting 2012 at Hyderabad.
- Attended Biology 2012 and beyond at CCMB Hyderabad in November 2012.

- Attended GI Immunology conference at Gurgaon India in January 2013.
- Delivered an invited lecture on 'A post translational point of view of Salmonella infection' at 16th ADNAT meeting at Hyderabad in 16-19th December 2012.

Dr. Saikat Bhattacharjee

- Attended the 3rd Conclave for Ramalingaswami Re-entry fellows organized by National Centre for Cell Science (NCCS), Pune- 12-14th September, 2013.

Dr. Tushar Kanti Maiti

- Delivered an invited lecture entitled "Molecular basis of Ubiquitin-C terminal Hydrolases function' in the International Symposium on Molecular Signaling organized by Visva Bharati, Santiniketan, February 18-21, 2013
- Delivered a keynote lecture entitled "Proton transfer reactions in biomolecules in the Asian International Symposium' organized by the Chemical Society of Japan, March 24, 2013, Japan.

Membership of Professional/ Academic Bodies/ Editorial Boards

Dr. Dinakar M. Salunke

- Member, Governing Council, National Brain Research Centre, Manesar
- Member, Governing Body, National Institute of Plant Genome Research, New Delhi
- Member, Governing Body, Translational Health Science & Technology Institute, Gurgaon
- Independent Director, Biotechnology Industry Research Assistance Council (BIRAC) Board
- Member, Commission on Biological Macromolecules, International Union of Crystallography
- Consultant, Commission on Synchrotron Radiation International Union of Crystallography
- Vice President, Indian National Science Academy
- Member, Council of Scientific Advisers, International Centre for Genetic Engineering and Biotechnology
- Member, Scientific Advisory Committee, Bose Institute, Kolkata
- Member, Scientific Advisory Committee, National Brain Research Centre, Manesar
- Member, Scientific Advisory Committee for Biosciences and Bioengineering Group, IIT Indore
- Chairman, INSA National Committee for International Union of Crystallography
- Member of Expert Committee on Fund for Improvement of S&T Infrastructure in Higher Educational Institutions (FIST), Department of Science & Technology
- Member, Apex Committee, Biotechnology Industry Partnership Programme
- Member, Finance Committee, National Brain Research Centre, Manesar
- Member, INSA Sectional Committee on Cell and Biomolecular Sciences
- Member, Committee of Experts for Planning Synchrotron Radiation Sources, RRCAT, Indore
- Chairman, North Eastern Region-Biotechnology Programme Monitoring Committee, DBT
- Member, Scientific Advisory Committee, Bose Institute, Kolkata
- Chairman, Management Board, Technology Advancement Unit, DBT

Dr. Prasenjit Guchhait

- Member of the Editorial Board for the Journal of Hypertension and Cardiology.
- Member of the Editorial Board for the World Journal of Hypertension.

Dr. Avinash Bajaj

- Member of the Editorial Board for the Journal Frontiers in Chemistry.

Dr. Tushar Kanti Maiti

- Distinguished Lectureship Award for the work on *Spectroscopy of Biomolecules* in the CSJ Asian International Symposium organized by The Chemical Society of Japan, March 21-24, 2013 Shiga Japan.

Distinctions, Honours and Awards

Dr. Sam J. Mathew

- Awarded the Wellcome Trust/DBT India Alliance Intermediate Fellowship for the proposal "The role of developmental Myosin Heavy Chain (MyHC) genes in skeletal muscle development, regeneration, homeostasis and disease".
- Awarded the Ramanujan Fellowship from the Department of Science and Technology (DST) for the proposal "Intrinsic signals that regulate skeletal muscle structure and function" (Declined)

Dr. Saikat Bhattacharjee

- Awarded Ramalingaswami Re-entry Fellowship of the Department of Biotechnology.

RCB Colloquia

RCB organized scientific colloquia inviting outstanding scientists namely Professor Angelo Azzi from Tufts University, Boston, and Professor Joel Sussman, Weizmann Institute of Science, ISRAEL.

Professor Angelo Azzi talked about the interesting genes (AMPK, PGC-1α, mTOR etc.) that regulate the lipid metabolism, hallmark condition of obesity. He described the association of these genes in various signaling pathways, which are involved in secondary disorders such as diabetes, hypertension, degenerative disorders. A depiction about how diet, physical exercise modulates the disease in mice models was presented. He also gave an important message about the therapeutic interventions on this gene regulation to treat the diseases, and showed the therapeutic potential of many natural products such as curcumin, resveratrol, quercetin in regulating the lipid metabolism.

Professor Joel Sussman made an interesting presentation on biochemical, structural, functional, and therapeutic aspects of acetylcholinesterase. Acetylcholinesterase an enzyme, is a prime therapeutic target for many peripheral and central cholinergic nervous system disorders. He showed his pioneering work with 3D- visualization of acetylcholine entry *via* hydrophobic path, binding in active pocket, and exit of catalyzed products. He showed the importance of differential charge and hydrophobic domain distribution of acetylcholinesterase. He also revealed the mechanism of actions of different reversible, irreversible inhibitors of AchE including recent Syria nerve gas (sarin) disaster with 3D-visuals. He then divulged the role of AchE inhibitors in Alzheimer's disease and drug design of new inhibitors.

Prof. Angelo Azzi delivering the colloquium lecture

Discussion during colloquium

Prof. Joel Sussman delivering the colloquium lecture

Workshops & Study Visits

RCB organized a hands-on workshop on *Proteopedia*-A Powerful Tool for Biomolecular Communication and a 3D Web Encyclopedia of Biomolecules on 30 September, 2013. Prof. Joel L. Sussman from the Israel Structural Proteomics Center (ISPC), Weizmann Institute of Science, Israel demonstrated the new resource, *Proteopedia* and its application in teaching and research. Scientists and students are now able to access 3D images of biomacromolecules underlying biological functions and disease, create their own pages, include 3D interactive scenes via a user friendly GUI for Jmol, and add text with hyperlinks to the interactive scenes in *Proteopedia*. 30 students/ post-doctoral fellows/scientists from RCB as well as other institutions/universities across the NCR regions benefited from the hands-on training imparted in the workshop.

Students attending the workshop on Proteopedia

Students from Department of Biotechnology, Kathmandu University, Nepal accompanied by a faculty member visited RCB on 11.03.2013. RCB faculty guided them in familiarizing the latest technological developments in the broad biomedical research and provided exposure to the facilities available at RCB. Students from Jayoti Vidyapeeth Women's University (JVWU), Jaipur visited RCB along with their 2 faculty members on 29.05.2013 to explore the on-going scientific activities at RCB. RCB Faculty provided an overview of ongoing work to the students. The students were also taken for tour around the laboratories.

Seminars Delivered by Visiting Scientists

Speaker	Title	Date
Joel L.Sussman The Israel Structural Proteomic Center, Weizmann Institute of Science	"Workshop on Proteopedia"-A Powerful Tool for Biomolecular Communication and a 3D Web Encyclopedia of Biomolecules	Sept. 30, 2013
Santi M. Mandal Central Research Facility, IIT Kharagpur, India	Future of Antibiotics: Getting Worse or Receiving Better Therapy?	Sept. 20, 2013
Arijit Roy Stony Brook University, Stony Brook, USA	Computing the relative stabilities and the per-residue components in protein conformational changes	Sept. 20, 2013
Dandamudi Usharani The Hebrew University of Jerusalem, Israel	Influence of the Protein Environment on the Mechanistic Function of Cytochrome P450 (CYP) Enzymes	Sept. 10, 2013
Rahul Das University of California Berkeley, USA	Structural basis for conformational coupling across the plasma membrane in activation of EGFR	Sept. 5, 2013
Mukesh Lodha Cold Spring Harbor Laboratory USA	Structural Epigenetic regulation of KNOX loci in differentiating Arabidopsis leaves	Aug. 30, 2013
Renu Mohan Cell Biology Department, Utrecht, Netherlands	Microtubules, Microtubule Associated Proteins (MAPs) and Cancer Therapy	Aug. 29, 2013
Karunakar Kar Pittsburgh Institute for Neurodegenerative Diseases TU Delft, University of Pittsburgh	Polyglutamine amyloids: aggregation mechanism and cytotoxicity	Aug. 26, 2013
Sandeep K. Sharma Biochemisches InstitutUniversitat Zurich, Switzerland	Probing the mechanisms of chaperone mediated protein folding.	Aug. 23, 2013
Deepti Trivedi Vyas University of Texas Medical Branch, Galveston, Texas, USA	Molecular Genetic Regulation of Synaptic Structure and Function	Aug. 21, 2013
Navratna Vajpai Protein Structure and Biophysics, AstraZeneca R&D, UK	Structural understanding of protein kinases inhibitory mechanism under solution conditions Receptor	Aug. 16, 2013

Speaker	Title	Date
Sabu Abraham Dept. of Cell Biology University College, London, UK	Novel Regulators of Retinal Vascular Disease	Aug. 6, 2013
Gautam V. Soni Kavli Institute of NanoScience TU Delft, The Netherlands	Nanopore Biophysics: From Gene Sequencing to Gene Silencing	July 8, 2013
Rakesh Kumar School of Biotechnology SMVDU, Katra, Jammu, India	MOF and histone H4 acetylation at lysine 16 are critical for DNA damage response and double-strand break repair	June 21, 2013
Deepti Jain National Centre for Biology Sciences, Bangalore	Regulation of Transcription Initiation in Prokaryotes: Structures and Mechanisms	June 19, 2013
Priyadarshini Chatterjee Children's Hospital Harvard Medical School, Boston, MA	Role of complement C4 in maintenance of B cell tolerance	June 17, 2013
Sairam Mallajosyula University of Maryland Baltimore, Maryland, USA	Protein Glycosylation: From Force Field Development to Applications	June 14, 2013
Manikandan Subramanian Columbia University Medical Center, New York	Dendritic Cell Efferocytosis-Mediated Antigen Acquisition is a Critical Mechanism for Cross-Presentation and Anti-Viral Immunity	May 9, 2013
Naeha Subramanian National Institute of Allergy and Infectious Diseases, Bethesda, USA	Innate Sensing, Inflammasomes and NLRs: New Insights Revealed by Imaging and Systems Approaches	April 23, 2013
Manvendra Singh Department of Cell, and Developmental Biology, University of Pennsylvania, Philadelphia, PA	Role of Semaphorin3D in Cardiovascular development and disease	April 16, 2013
Josef Prchal	Hypoxia & Erythropoiesis in Health and Disease	April 05, 2013
Mahavir Singh Department of Biochemistry Univ. of California Los Angeles, USA	Structural basis for telomerase RNA recognition and RNP assembly by the holoenzyme La family protein p65	March 5, 2013

Speaker	Title	Date
Tina Mukherjee Department of Molecular, Cell, and Developmental Biology, Univ. of California, Los Angeles, CA	Systemic regulation of hematopoietic progenitors maintenance during Drosophila blood development	March 5, 2013
Malik Keshwani HHMI, University of California, San Diego	cAMP dependent protein kinase A (PKA): New insights from an old kinase	March 4, 2013
Paras Anand ST. JUDE Children's Hospital, Memphis, TN	Nod-Like Receptors in Pathogen Recognition and Host Defense	Feb. 28, 2013
Bani Kanta Sarma Department of Chemistry The Scripps Research Institute Florida	Rapid construction and high-throughput screening of novel combinatorial libraries to identify bioactive molecules	Feb. 25, 2013
James Premdoss Clement Chelliah Scripps Research Institute, Jupiter, FL	Pathogenic SYNGAP1 haploinsufficiency impairs cognitive development by disrupting the maturation of dendritic spine synapses	Feb. 22, 2013
Amitabha Majumdar Stowers Institute for Medical Research, Kansas City, Missouri	The role of a self-sustaining amyloidogenic protein in persistence of memory	Feb. 15, 2013
Jaspreet Khurana Princeton University, USA	Non-coding RNAs as Genome Sculptors	Feb. 15, 2013
Tania Bose Stowers Institute for Medical Research, Kansas City, Missouri	Cohesin promotes rDNA transcription and protein translation	Feb. 14, 2013
Ankit Gupta Univ of Massachusetts, Worcester MA	Gene Targeting by ZFNs and TALENs in Zebrafish	Feb. 14, 2013
Anoop Sindhu University of Saskatchewan, Canada	Applications of RNAi: A New Generation Technology for Pest Control.	Jan. 21, 2013
Babul Jha Lerner Research Institute, Cleveland Clinic Cleveland, Ohio USA	Therapeutic Implications of Targeting OAS/RNase L System against Cancer and Viral Infections.	Jan. 04, 2013
Sangita Sinha Department of Chemistry and Biochemistry NDSU, Fargo, ND	Targeting gamma-herpesvirus Bcl-2 inhibition of autophagy and apoptosis.	Dec. 13, 2012

Speaker	Title	Date
Kanika Bajaj Pahuja University of California Berkeley, USA	Understanding determinants of protein trafficking : A new perspective on COPII vesicular transport.	Dec. 5, 2012
Sunil Raghav Swiss Federal Institute of Technology (EPFL)Lausanne	Integrative genomics approach for a systems level understanding of the regulatory networks underlying fat cell differentiation	Nov. 26, 2012
Saravanan Matheshwaran European Molecular Biology Laboratory, Heidelberg, Germany	Interactions between the nucleosome histone core and Arp8 in the INO80 Chromatin Remodeling Complex	Nov. 7, 2012
Sharmistha Sinha Iowa State University, USA	Molecular Tweezers: Novel Therapeutic Strategies for the Treatment of Dementia Related Diseases	Oct. 18, 2012
Sangita Roy Dept. of Pure and Applied Chemistry, University of Strathclyde Glasgow, UK	Responsive Peptide Hydrogels for Biotechnological applications	Oct. 17, 2012
Chetana Sachidanandan IGIB Delhi	Zebrafish: A Versatile Vertebrate Model	Oct. 16, 2012
Rakesh Mishra CCMB, Hyderabad	Seminar	Oct. 16, 2012
Rupinder Kaur CDFD, Hyderabad	The Yeast Saccharomyces cerevisiae: An Awesome Model System	Oct. 15, 2012
Kavita Babu IISER, Mohali	Synaptic plasticity at the C. elegans Neuromuscular Junction	Oct. 14, 2012
Sandhya Koushika Department of Biological Sciences TIFR, Mumbai	Using the C. elegans Model to Understand in vivo Synaptic Vesicle Transport in Neurons	Oct. 14, 2012
Rohit Joshi CDFD, Hyderabad	Fly Biology 101	Oct. 13, 2012
K Subramaniam Dept. of Biological Sciences and Bioengineering, IIT, Kanpur	The Balancing Act of Mitosis-Meiosis Decision	Oct. 13, 2012
L S Shashidhara IISER, Pune	Functional genomics and drug discovery: use of alternative model organisms	Oct. 12, 2012
Kausik Chakraborty IGIB, Delhi	Genetic landscape of the unfolded protein response of endoplasmic reticulum	Oct. 12, 2012

Extramural Activities & Funding

Extramural Activities

In pursuit of achieving top class biotechnology education and training in a research milieu at the interface of multiple disciplines, RCB is engaged in a wide range of extramural activities which will directly or indirectly facilitate fulfillment of its core mandate. The activities include an array of international as well as national level participations with critical responsibilities.

Biotech Science Cluster Cell

RCB is engaged in facilitating the establishment of NCR Biotech Science Cluster, Faridabad (BSCF), in partnership with Translational Health Science and Technology Institute (THSTI), National Institute of Immunology (NII), National Plant Genome Research Institute (NIPGR) and National Brain Research Institute (NBRC). As part of these activities it has have been anchoring the establishment of physical infrastructure in the Faridabad campus.

Advanced Technology Platform Centre

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

Technology Advancement Unit

RCB is involved in managing the Technology Advancement Unit, An Indo-Swiss Programme of DBT. As part of its multi-dimensional role of an inter-institutional coordinator, RCB has hosted the TAU and has provided administrative facilities for TAU. RCB provides the administrative, institutional and statutory support to TAU for expansion of the activities to accomplish the desired objectives as per mutual interest of both the parties.

Bio-Incubator

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

Regional and International Networking

Under the Indo-Japan collaboration programme of the Department of Biotechnology, RCB has been involved in the setting up of a AIST high-end imaging facility. RCB will be housing the Japanese lab and will be undertaking the collaborative research of mutual interest. As a networking initiative with the scientists located in the South-Asian regions, RCB faculty have participated in the Second Summit of the South Asian Science Academies organized at INSA, New Delhi and interacted with them. Further modalities are being worked out to enhance scientific linkages with the member institutions in the region. Students from Department of Biotechnology, Kathmandu University, Nepal accompanied by a faculty member visited RCB on 11.03.2013. RCB faculty guided them in familiarizing the latest technological developments in the broad biomedical research and provided exposure to the facilities available at RCB. Executive Director, RCB is a member of the Scientific Advisory Committee of a newly established UNESCO Category II Centre in biotechnology for African region at the University of Nigeria, Nsukka. RCB is keenly engaged in providing logistics support to this new centre in Africa. Likewise, the Ministry of Science & Technology, Nepal is planning to initiate novel programmes in biotechnology, for which association of RCB has been sought. RCB would be imparting short-term training programmes for Visiting Scientists from the countries in the region and other developing countries. The programme for international visiting scientists has already been initiated.

Extramural Funding

The following extramural R&D grants have been received by the faculty at this Centre:

- Engineering of Nanomaterials and their interactions with DNA and Cell Surface: *Dr. Avinash Bajaj; Rs. 73.00 lakhs (DST)*
- Engineering of Nanomaterials for Combination Cancer Therapy: *Dr. Avinash Bajaj; Rs. 99.75 lakhs (DBT)*
- Investigation of ligand induced activation mechanism of Adenosine 2A receptor by ATR-FTR Spectroscopy Indian Japan Cooperation Science programme: *Dr. Tushar K Maiti; Rs. 2.52 lakhs (DBT)*
- Collaboration for translation and clinical research between Translation Health Science and Technology Institute, National Brain Research Centre, Regional Centre for Biotechnology and Gurgaon, Civil Hospital: *Dr. Dinakar M Salunke; Rs. 79.05 lakhs (DBT)*
- Molecular Basis for Silencing of the Spindle Assembly Checkpoint: *Dr. Sivaram Mylavarapu; Rs. 62.02 lakhs (DBT)*
- Elucidating inositol-dependent signalling routes of effector-triggered immunity for identifying new approaches for engineering crop resistance against diverse pathogens: *Dr. Saikat Bhattacharjee; Rs. 82.00 lakhs (DBT)*
- Understanding Salmonella-mediated alterations in host SUMOylation: implications in infection and inflammation: *Dr. C.V. Srikanth; Rs. 3.28 Cr (DBT/ Wellcome Trust India Alliance)*
- Role of developmental myosin heavy chains in skeletal muscle development, regeneration, homeostasis and disease: *Dr. Sam J Mathew; Rs. 3.10 Cr (DBT/ Wellcome Trust India Alliance)*

Infrastructure & Development

Administrative Activities

During the year ending 31.3.2013, the position of staffing in the Centre was enhanced to keep up with the growing needs of the Academic, Research and Technical activities by recruitment of staff with expertise in areas of Finance, Administration, and Stores and Purchase on contractual basis. The processes and procedures for conduct of the administrative, financial, and engineering and inventory management activities at the Centre were undertaken seamlessly to enhance the functioning of the centre. It has also provided adequate service support to 'Establishment of the Advanced Technology Platform Centre" at 181, Udyog Vihar Phase I, Gurgaon and also to the "Technology Advancement Unit": an initiative of the DBT.

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

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

Interim Laboratories in NCR, Gurgaon

The Interim Laboratories at 180, Udyog Vihar, Phase I, Gurgaon NCR are furnished with state of art equipments, instruments and facilities like Nuclear Magnetic Resonance Spectrometer (NMR), Advanced Proteomics Facility (MASS SPECTROMETRY), Surface Plasmon Resonance, Atomic Force Microscope – (BIO-AFM), Protein/ Peptide Sequencer, FPLC protein purification system, Dispenser 4 Crystallisation, CD Spectropolarimeter, Flow Cytometer, Workstations (8 Nos.), Isothermal Titration Calorimeter, Floor Ultra Centrifuge, Fluorescence 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 detector., Tissue Homogenizer, Cold Rooms, Refrigerated Centrifuge, Refrigerated Centrifuge, MCT Detector and Nano Spectrophotometer.

A Seminar Hall with state of art facilities has been established that can accommodate over 150 participants. The recently established seminar hall will cater to the requirement of RCB and also sister institutes in the vicinity. This is in addition to the regular classroom facility and conference room that are already equipped with state-of-art facilities including the video conferencing facility.

Status of the Permanent Campus at Faridabad

The permanent Campus of RCB as part of the NCR-BSC at Faridabad initiated as Phase-I of construction, consisting of the laboratory buildings, is nearing its completion. The process of installation of supporting infrastructural services and furnishing of the labs is currently being undertaken. The permanent campus of RCB is expected to be operational by the end of 2014. The timely initiation of the construction activity and close monitoring of the progress of work has resulted in successfully setting up of the infrastructure at Faridabad.

Simultaneously, the construction activity for the 4th Wing of the RCB Laboratory hostels for students and housing for faculty is also scheduled to commence early 2014 as part of the Phase-I Extension. Since RCB has also been entrusted with the task of setting up the Advanced Technology Platform Centre & the Bio-incubator, the construction of the building to cater to the requirements of these two centres has also been initiated.

With continued financial support from DBT and other relevant agencies like the THSTI, NII, NBRC and NIPGR participating in the NCR Biotech Science Cluster, it is expected that the project will mature into one of its kind in the world.

Construction at Faridabad site

Financial Statements

LALIT GUPTA & ASSOCIATES
CHARTERED ACCOUNTANTS

AUDITOR'S REPORT

We have audited the attached Balance Sheet of REGIONAL CENTRE FOR BIOTECHNOLOGY, 180, Udyog Vihar Phase-I, Gurgaon – 122016 as on 31st March 2013 and annexed Income & Expenditure Account and Receipts & Payments Account for the year ended on that date with the books of accounts and vouchers maintained by the Centre and report as under:-

1. That the Centre's Balance Sheet, Income & Expenditure Account and Receipts & Payment Account are in agreement with the books of accounts.
2. We conducted out audit in accordance with auditing standards generally accepted in India. Those Standards require that we plan and perform the audit to obtain reasonable assurance about whether the financial statements are free of material mis-statement. An audit includes examining, on a test basis, evidence supporting the amount and disclosures in the financial statements. An audit also includes assessing the accounting principles used and significant estimates made by the management, as well as evaluating the overall financial statement presentation. We believe that our audit provides a reasonable basis for our opinion.
3. Subject to Accounting Policies and Notes on Accounts as per Schedule-10, in our opinion and to the best of our information and according to the explanation give to us, the said accounts give a true and fair view:

- In the case of Balance Sheet of the State of Affairs of the Centre as at 31st March, 2013 and
- In the case of Income & Expenditure Account of the Centre during the period ended on that date.

For **LALIT GUPTA & ASSOCIATES**
Chartered Accountants

Sd/-
(PAWAN GARG)
Partner
Membership No. 095469

Place : Gurgaon
Date : 27th September 2013

REGIONAL CENTRE FOR BIOTECHNOLOGY
BALANCE SHEET AS AT 31st MARCH, 2013

Amount
(In Rs.)

CORPUS / CAPITAL FUND AND LIABILITIES	Schedule		Current Year		Previous Year
CORPUS / CAPITAL FUND	1	-	164,670,018.00	-	176,791,796.00
RESERVES AND SURPLUS	2	-	415,012.00	-	415,012.00
CURRENT LIABILITIES AND PROVISIONS	3	-	156,042,313.00	-	89,386,934.00
BIOTECH SCIENCE CLUSTER (BSC)	3	-	1,055,475,225.00	-	747,069,813.00
TOTAL			**1,376,602,568.00**		**1,013,663,555.00**
ASSETS				-	
FIXED ASSETS	4	-	126,310,026.00	-	97,765,990.00
FUNDS IN SHORT TERM DEPOSITS	5	-	76,540,000.00	-	128,626,669.00
CURRENT ASSETS, LOANS ADVANCES ETC.	5	-	136,180,369.00	-	55,737,161.00
BIOTECH SCIENCE CLUSTER (BSC)	5		1,037,572,173.00		731,533,735.00
a. Capital Work in progress			844,482,114.00		329,692,123.00
b. Advance to BSC construction			181,134,530.00		242,536,713.00
c. Funds in short term deposits			3,400,000.00		153,883,608.00
d. Accrued interest & TDS			8,555,529.00		5,421,291.00
TOTAL			**1,376,602,568.00**		**1,013,663,555.00**
SIGNIFICANT ACCOUNTING POLICIES					
CONT. LIABILITIES & NOTES ON ACCOUNTS					

AS PER OUR SEPARATE REPORT
OF EVEN DATE ATTACHED
Lalit Gupta & Associates
CHARTERED ACCOUNTANTS

Sd/-	Sd/-	Sd/-
BIJU MATHEW	**DINAKAR M SALUNKE**	**PAWAN GARG**
SENIOR MANAGER (A&F)	**EXECUTIVE DIRECTOR**	**PARTNER**
		Lalit Gupta & Associates

REGIONAL CENTRE FOR BIOTECHNOLOGY

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31st MARCH, 2013

Amount (in Rs.)

Income	**Schedule**	**Current Year**	**Previous Year**
Grants/Subsidies	6	120,570,621.00	81,348,251.00
Fees/Subscriptions	7	92,200.00	225,809.00
Interest on investments on fixed deposits	7	2,620,044.00	2,629,832.00
Deferred Income-Fixed Assets		71,021,778.10	42,114,814.00
TOTAL (A)		**194,304,643.10**	**126,318,706.00**
EXPENDITURE			
Establishment Expenses	8	27,373,725.00	20,713,811.00
Other Administrative Expenses etc.	9	95,909,140.00	63,490,081.00
Depreciation (Net Total at the year end-corresponding to schedule 4)		71,021,778.10	42,114,814.00
TOTAL(B)		**194,304,643.10**	**126,318,706.00**
Balance being excess of Income Over Expenditure (A-B)		-	-
Transfer to special Reserve(Specify each)		-	-
Transfer to /from General Reserve		-	-
BALANCE BEING SURPLUS (DEFICIT) CARRIED TO CORPUS/CAPITAL FUND		-	-

AS PER OUR SEPARATE REPORT
OF EVEN DATE ATTACHED
LALIT GUPTA & ASSOCIATES
CHARTERED ACCOUNTANTS

Sd/-	Sd/-	Sd/-
BIJU MATHEW	**DINAKAR M SALUNKE**	**PAWAN GARG**
SENIOR MANAGER (A&F)	EXECUTIVE DIRECTOR	PARTNER Lalit Gupta & Associates

Institutional Information

Board of Governors

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

Programme Advisory Committee

1.	**Prof. Angelo Azzi** Tufts University, 711 Washington ST. Boston MA 02111, USA	Chairman
2.	**Prof. K. VijayRaghavan** Chairman, Board of Governors, RCB and Secretary Department of Biotechnology, MoS&T, Govt. of India, New Delhi	Member
3.	**Dr. Satyajit Rath** Sr. Scientist, National Institute of Immunology Aruna Asaf Ali Marg, JNU Campus, New Delhi	Member
4.	**Prof. K. Veluthambi** School of Biotechnology Madurai Kamaraj University, Madurai	Member
5.	**Mr. S. Sinha** Dept. of Biotechnology, Ministry of Science and Technology Govt. of India, New Delhi	Member
6.	**Dr. K.V.S. Rao** International Centre for Genetic Engineering and Biotechnology New Delhi	Member
7.	**Dr. G.B. Nair** Executive Director Translational Health Science & Technology Institute, Gurgaon	Member
8.	**Prof. Shankar Subramaniam** Department of Bioengineering Department of Chemistry & Biochemistry, University of California at San Diego, USA	Member

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

10. **Prof. Jeongbin Yim** — Member
School of Biological Sciences, Seoul National University
Seoul, 151-742, Korea

11. **Prof. R Venkata Rao** — Member
Vice Chancellor
National Law School of India University, Bangalore

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

13. **Prof. M.K. Bhan** — Special Invitee
National Science Professor
Indian Institute of Technology, Delhi

14. **Prof. Subrata Sinha** — Special Invitee
National Brain Research Centre
NH-8, Nainwal Mode, Manesar-122050

15. **Prof. T P Singh** — Special Invitee
Department of Biophysics
All India Institute of Medical Sciences
Ansari Nagar, New Delhi

16. **Prof. Shinjini Bhatnagar** — Special Invitee
Translational Health Science & Technology Institute
496, Udyog Vihar, Phase-III, Gurgaon

17. **Prof. Joel Sussman** — Special Invitee
Department of Structural Biology
The Weizmann Institute of Science
Rehovot, Israel 76100

18. **Dr. Haseena Khan** — Special Invitee
Visiting Professor, Faculty of Life Sciences & Biology
South Asian University, Akbar Bhawan
Chanakyapuri, New Delhi

Executive Committee

1. **Dr. Dinakar M. Salunke** — Chairman
Executive Director, Regional Centre for Biotechnology,
Gurgaon

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

3. **Mr. S. Sinha** — Member
Adviser, Dept. of Biotechnology,
Govt. of India, New Delhi

4. **Mr. Amit Khare** — Member
Joint Secretary (IC), Dept. of Higher Education
Ministry of Human Resource Development
Govt. of India, New Delhi

5. **Mr. T S Tirumurti** — Member
Joint Secretary, UNESCO Division
Ministry of External Affairs
Govt. of India, New Delhi

6. **Dr. Satyajit Rath** — Special Invitee
Sr. Scientist, National Institute of Immunology
Aruna Asaf Ali Marg, JNU Campus, New Delhi

Finance Sub-Committee

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

Staff

Scientific Faculty

Dr. Dinakar M Salunke
Executive Director

Dr. Prasenjit Guchhait
Associate Professor

Dr. Sivaram V. S. Mylavarapu
Asstt. Professor

Dr. Avinash Bajaj
Asstt. Professor

Dr. Vengadesan Krishnan
Asstt. Professor

Dr. Tushar Kanti Maiti
Asstt. Professor

Dr. Chittur V. Srikanth
Asstt. Professor

Dr. Sam Jacob Mathew
Asstt. Professor

Dr. Saikat Bhattacharjee
Asstt. Professor

Dr. Priyadarshini Chatterjee
Asstt. Professor

Prof. Falguni Sen
International Adjunct Faculty

Academic Management

Mr. Biju Mathew
Registrar (I/C)

Ms. Vaishali Mangla
Doc. Asstt.

Mr. Deepak Kumar
Doc. Asstt.

Administration & Finance

Mr. Biju Mathew
Senior Manager (A&F)

Dr. Sanjay Kumar
Staff Officer to Executive Director

Mr. V.M. S. Gandhi
Administrative Officer

Mr. Sanjeev Kumar Rana
Jr. Mgmt. Asstt.

Mr. Sudhir Kumar
Jr. Mgmt. Asstt.

Engineering

Mr. Ramesh Kumar Rathore
Assistant Engineer

Technical Assistants

Mr. Madhava Rao Medikonda
Mr. Suraj Tewari
Mr. Vijay Kumar Jha
Ms. Vishakha Chaudhary
Mr. Atin Jaiswal
Mr. Ramesh Chandiramouli

Consultants

Mr. C.L. Raina
Finance
Mr. Shyam Sunder Budhwar
Engineering
Mr. Ratindra Nath Chatterjee
Junior Consultant
Dr. Nirpendra Singh
Technical
Dr. Ujjaini Das Gupta
Technical

Young Investigators

Dr. Jasmita Gill
Dr. Alka Dwevedi
Dr. Smriti Verma
Dr. Sushmita Bhattacharya
Dr. Susheela Kushwaha
Dr. Masum Saini
Dr. Sheetal Chawla
Dr. Himanshu Arora
Dr. Megha Kumar

Senior Research Fellows

Ms. Abha Jain
Mr. Sagar P. Mahalle
Mr. Vedagopuram Sreekanth
Mr. Harsh Kumar

Junior Research Fellows

Ms. Harmeet Kaur
Ms. Pranita Hanpude
Mr. Pergu Rajaiah
Mr. Gowtham Kumar Annarapu
Mr. Somanath Kundu
Ms. Gayatree Mohapatra
Ms. Samta Marwaha
Mr. Roshan Kumar
Mr. Amit Sharma
Ms. Priyanka Chaurasia
Ms. Sarita Chandan Sharma
Mr. Salman Ahmad Mustfa
Ms. Rashi Singhal
Ms. Kavita Yadav

Research Associates / Post Doctoral Fellows

Dr. Manish Singh
Research Associate (Project)
Dr. Sandhya Bansal
DBT Research Associate
Dr. Mukesh Kumar
Post Doctoral Fellow

Project Assistants

Mr. Priyanshu Bhargava
Ms. Swati Jain

Scientific Officer

Mr. Suneel Prajapati
Scientific Officer (Project)

Trainees

Mr. Animesh Kumar
Ms. Anju Rathi
Ms. Geeta Sandhu
Mr. Avinash Gupta
Ms. Shivangi Dubey
Mr. Anirudh Srinivas M.
Ms. Mallika Tiwari
Mr. Abhinav Kurumaddali
Ms. Natasha Mathur
Mr. Vinodh Kumar
Ms. Divya Gahlot
Ms. Nisha Tyagi
Ms. Divya Grover
Ms. Manasa Chaudhary
Mr. Yogesh Sharma

Snapshots

Flag hoisting on 15th August

Drawing Competition on 15th August

Fancy Dress Competition on 15th August

Tree plantation at the Faridabad Site

Hindi Kavita Competition

Discussion with the Colloquium Speaker

Discussion on Poster during PAC meeting

Designed and Printed by:
AFE Consultants Private Limited, Aravali House, 431/D-22, Chhatarpur Hills, New Delhi-110074, India

About RCB

The Regional Centre for Biotechnology (RCB) is an institution of education, training & research established by the Department of Biotechnology, Government of India under an agreement with UNESCO. Designed to be a Centre of Excellence in Biotechnology with intimate contributions from the countries of the region and academic institutions from the rest of the world, RCB aims to be platform for innovation, enterprise, and industrial development. As an institution, RCB is beneficial to all countries in the region including India in carrying out biotechnology research of highest caliber and developing knowledge-rich highly skilled human resource. Biotechnology being a global discipline, the partnerships are as much within as they are across international boundaries. Association with UNESCO enhances the possibilities at RCB to provide world class education, conduct quality research and foster global cooperation.

UNESCO
United Nations Educational, Scientific and Cultural Organization

REGIONAL CENTRE FOR BIOTECHNOLOGY
an institution of education, training and research
Established by the Dept. of Biotechnology, Govt. of India
Under the Auspices of UNESCO
180 Udyog Vihar Phase 1, Gurgaon - 122016, India