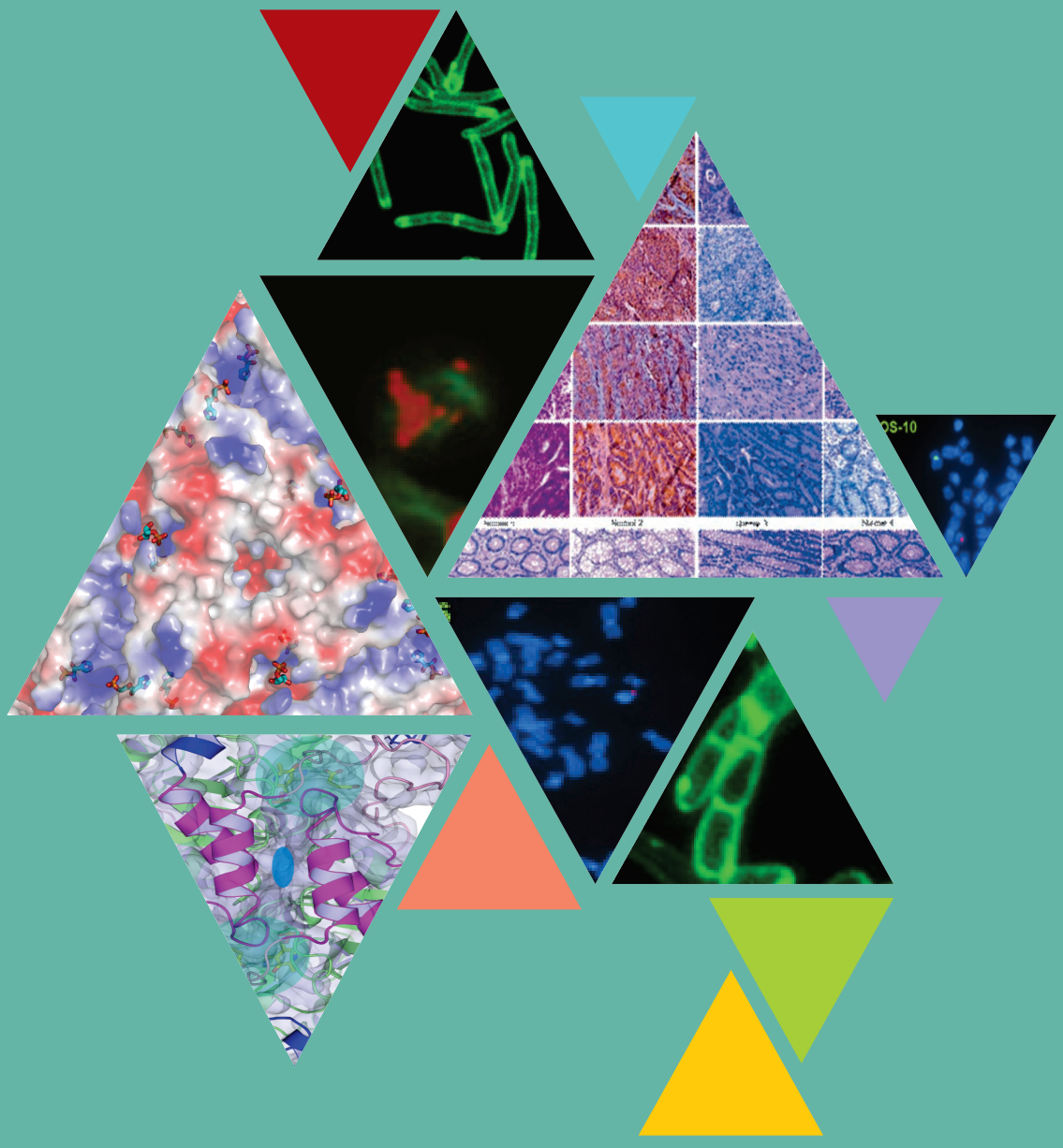
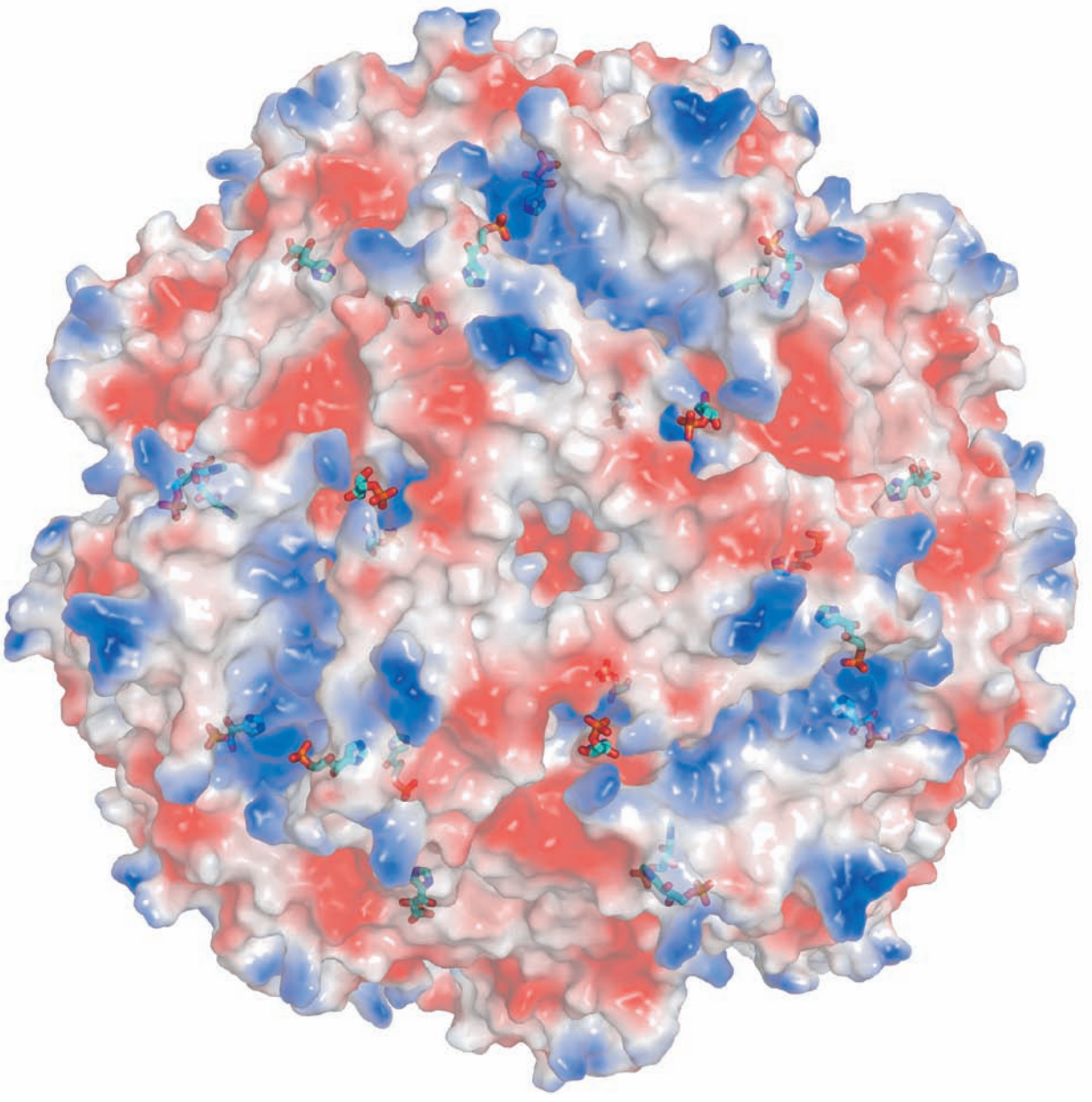
Annual Report
2015-2016

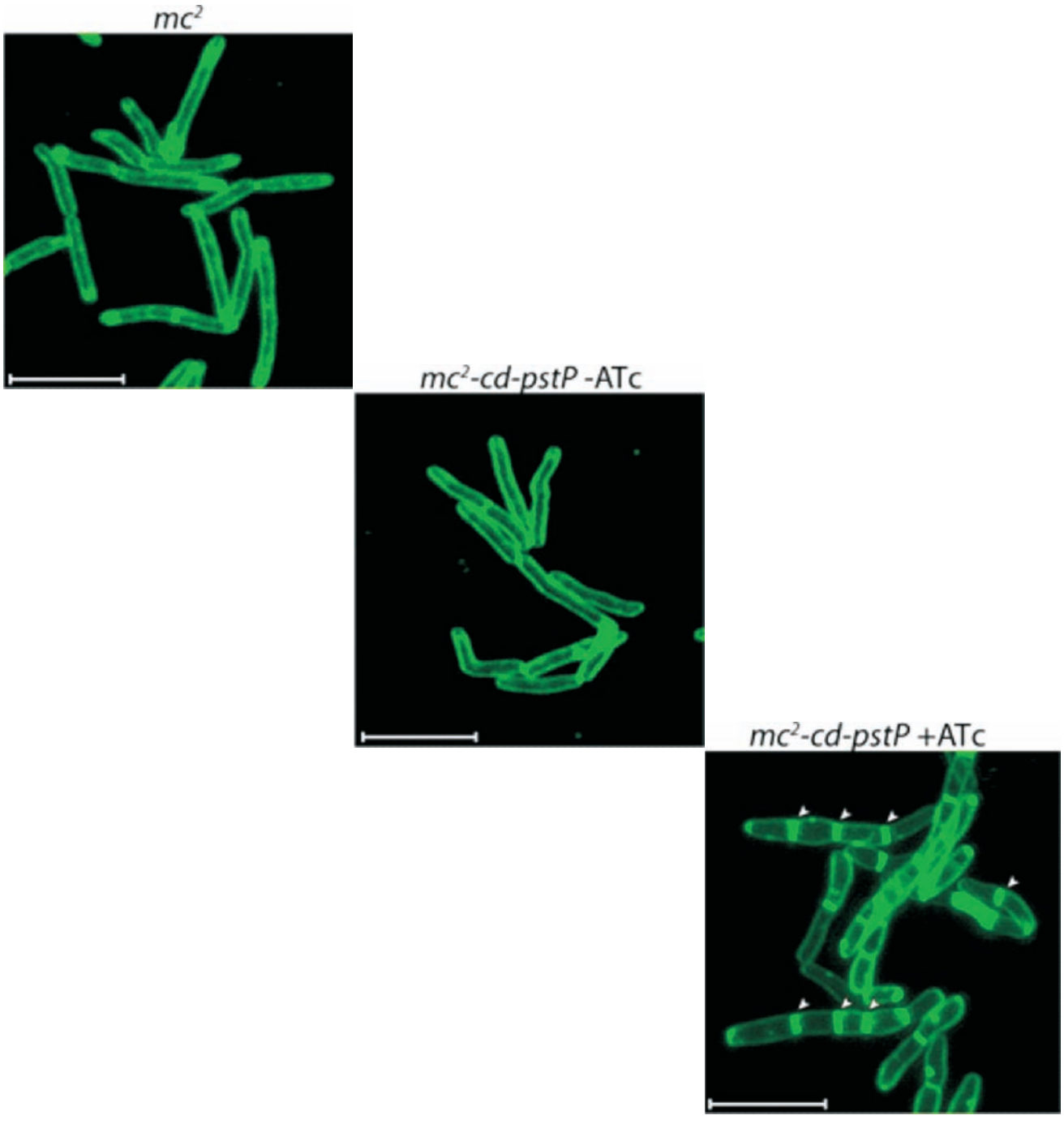


National Institute of Immunology



Electrostatic potential surface representation of HisB from Mycobacterium tuberculosis with bound substrate molecules in stick model

CONTENTS



Flourescence microscopy analysis. FM64 labelling and microscopy of *mc²* and *mc²::cd-pstP* (conditional mutant) strains grown in absence or presence of ATc was performed as described in methods. Arrows indicate the presence of septa no arrows are visible to me. Scale bar: 5 µm

MANDATE OF THE INSTITUTE

- To undertake, aid, promote, guide and co-ordinate research of high caliber in basic and applied immunology.
- To carry out research for development of new vaccines and immunological reagents for communicable diseases.
- To develop immunological approaches for regulation of male and female fertility.
- To interact with industry for manufacture of vaccines and immunological reagents.
- To organise postgraduate courses, workshops, seminars, symposia and training programmes of a specialized nature in the field of immunology, vaccine development and related areas.
- To organise training programmes for technicians in immunological methods and related techniques.
- To establish affiliation with recognised universities and institutions of higher learning for the purpose of enabling research scholars to register for postgraduate degrees.
- To serve as a national reference centre for immunology and to provide consultancy services to medical and veterinary institutions, public health agencies and industries in the country.
- To provide and promote effective linkages on a continuing basis between various scientific and research agencies/laboratories and other organisations working in the country in the field of immunology, vaccine development and related areas.
- To collaborate with foreign research institutions, laboratories and other international organisations in fields relevant to the objectives mentioned above.

FOREWORD

It is a great pleasure to present the achievements of the National Institute of Immunology for a very productive year. I congratulate all staff members for making this possible. The research areas of the institute encompass immunology and other related disciplines of life sciences spanning several overlapping domains of modern biology including biochemistry, molecular biology, cell biology and structural biology. During the year, the community of scientists with expertise in diverse areas has made mark in their respective turfs enriching the relevant areas. The scientific contributions of the institute would not have been possible without the enthusiastic participation of doctoral and post doctoral fellows. While twenty five students received their doctoral degrees, the institute also housed short and long term trainees in different research areas. The endeavours of the scientists were effectively supplemented by the solid functioning of the technical, administrative and other support staff of the institute.

The institute contributed to the translational space through the development of a vaccine for cervical cancer. This vaccine is up for clinical trials in late stage cervical cancer patients which is being conducted by the Cancer Institute at Adyar in Chennai. Another contribution is the transfer of technology of "Feeding strategy of high cell density fermentation" developed at the institute to Imgenex, India Private Limited.

The research areas at the institute are grouped in four broad areas, namely, infection and immunity, molecular design, gene regulation and reproduction and development. Within the ambit of these areas, advanced research in modern biology is being carried out using novel tools and advances to generate knowledge. I highlight below some of the important achievements during the year.

Multiple groups are involved in infectious disease research ranging from viral, bacterial and parasitic diseases. Research on HIV-1 replication involving proteins Tat and Rev revealed that Rev decreases Tat protein indirectly by down-regulating NQO1 levels. The findings have implications in understanding HIV-1 gene expression and latency. Tuberculosis is a huge problem in the country and multiple laboratories at NII are involved in working on the biology of the pathogen. Studies with *M. tuberculosis* showed that depletion of $GlmU_{Mtb}$ (N-acetyl-glucosamine-1-phosphate uridyltransferase), a bi-functional enzyme engaged in the synthesis of metabolic intermediates, in mice with a well-established infection, results in

irreversible bacterial death. $GlmU_{Mtb}$ is indicated as a promising target for therapeutic intervention and therefore, its inhibitor Oxa33 could be taken up as a lead molecule. Kala-azar or leishmaniasis caused by the pathogen *Leishmania donovani*, which is opportunistic in nature, shows Rab1 localization to the Golgi. Over expression of LdRab1 inhibits the secretion of secretory acid phosphatase by *Leishmania* and also blocks the secretion of Ldgp63. This shows the conserved nature of Rab1-regulated secretory pathway. However, hemoglobin receptor trafficking is Rab1-independent. The area of gut infection is important and in view of existing developments much needs to be worked upon. Research in the area of NF-κB signaling in gut infections showed that cell-differentiating cues provide signals in the niche of the tissue microenvironment and is able to crosstalk with the pathogen-responsive signaling circuitry. Such crosstalk supports the inflammatory response of NF-κB and is critical for lessening gut-infections.

Important contributions were made in the area of cell signaling in cancer biology. Findings show that for Bloom syndrome protein (BLM) Thr182 phosphorylation is important. It regulates BLM turnover during mitosis and also helps to preserve chromosomal stability. Other related studies show that accessory mitochondrial replication helicase RECQL4 inhibits the invasive step required for neoplastic transformation process. A study from another laboratory reported the role of deacetylase activity in sirtuin 6 tumor suppressor functions where it was shown that sirtuin6 mediated deacetylation of PKM2 (pyruvate kinase M2) results in loss of its oncogenic potential. Investigations on p73 interactome show ubiquitin ligase TRIM28 as a molecule determining p73 stability and MED15 as the coactivator that encourages anti-metastatic functions of p73.

Actively dividing cells show number of stages known collectively as the cell cycle. Research on neuronal cell-cycle reveals a novel mechanism involved in cell cycle re-entry and apoptosis in neurons. Alzheimer-related beta amyloid peptide cause deregulation of microRNA-34a through degradation of its transcriptional regulator TAp73. As a result, expression of cyclin D1- a target of miR34a- is elevated leading to re-entry into the cell cycle. Another novel discovery shows the ability of hSSB1-INTS3 protein complex to sense DNA damage followed by recruitment of ATR initiating the checkpoint-signaling cascade. Studies on insulators suggest existence of varied mechanisms of insulator action manifested concurrently or selectively and depend on the genomic context along with lessening of enhancer activity. The above studies resulted in publications of high impact along with many other papers in journals of relevance thus, contributing a substantial amount of new knowledge.

Collaborative endeavours are pillars of progress for scientific institutions. Two collaborative efforts with organizations outside the country has progressed well, three Queen Elizabeth fellows spent time at the institute during the summers receiving training in different areas. Scientists from the Singapore Immunology Network visited the institute to start collaborative dialogues. Two centres of excellence involving collaborations from within and outside the institute to be housed at NII were funded. While the institute nurtures many collaborative efforts all across the world, it is also a founder partner in a programme to develop multi-institutional collaborative effort which is coming up as a Biotech Science Cluster at the

National Capital Region. NCR Biocluster activities picked up pace during the year with registering of the NCR Biotech cluster as a society. NII along with the other collaborative institutes would now be able to take strides into giant collaborations sharing facilities with others.

NII attaches significant importance to its connectivity with society. The science and society programme of Science Setu had another successful year. Four more colleges from Delhi University joined the efforts and two lectures by prominent speakers were arranged in Hindu College and Zakir Husain College. The talks on stem cells and forensic sciences were very well received by the students who enthusiastically took part in the essay competitions and quiz programmes that were arranged along with the lectures. Fifty students were trained during the year in the laboratories with twenty faculty teaching at the various undergraduate colleges. I am happy that what started as a small effort by NII has flowered and other institutions are also starting the programme. This will immensely contribute to exposure of the undergraduate students to a laboratory life which is an important step in helping the students to make a career choice in science.

NII alumni are the pride of the institute. During the past year an effort was undertaken to reach out to alumni to connect to the current students. An enthusiastic response resulted in a mini symposium titled "Molecular and cellular biology beyond the Rubicon: graduating perspectives". A sizeable number of alumni took part in this event and a great interaction with faculty and current students took place. I am sure this will go a very long way in creating future collaborations. Several student welfare programmes were undertaken during the year. A career development workshop for third and fourth year students was conducted with a view to expose the students on the topics of team building, independent thinking, leadership values etc. A coordinated effort from the students and faculty resulted in the launch of the placement cell for the students. This will facilitate the efforts of students to explore engagement possibilities in the academia and industry once they complete their doctoral studies.

A number of MoUs were signed between NII and other organizations to take forward collaborative initiatives. MoUs were signed between *S*ingapore Immunology Network (Biomedical Sciences Institutes); University of Toronto (Canada); Institute of Liver and Biliary Sciences, Delhi; CSIR-IMTECH, Chandigarh; Birla Institute of Science-Pilani, Hyderabad; National Institute for Research in Reproductive Health (NIRRH), Mumbai and the Department of Pharmaceutical Sicences, Dr. Harisingh Gour Vishwavidyalaya, Sagar for collaborative research.

I congratulate the scientists who received recognitions in the form of awards, appointments to professional bodies and election to the various academies in India and abroad. These awards are listed separately in the annual report. I also welcome three new colleagues who joined our team of scientists, Dr. Vijay Yadav, Dr. Nimesh Gupta and Dr. Ankita Varshney. I would like to express my gratitude to four scientists who superannuated during the reporting period, Drs. Satish Gupta, Rajni Rani, Dinakar Salunke and Sher Ali. Their invaluable contributions to the institute over the years have helped NII to sustain and improve its excellence. Manager administration Shri Girish Bharihoke also superannuated after a long service period at NII.

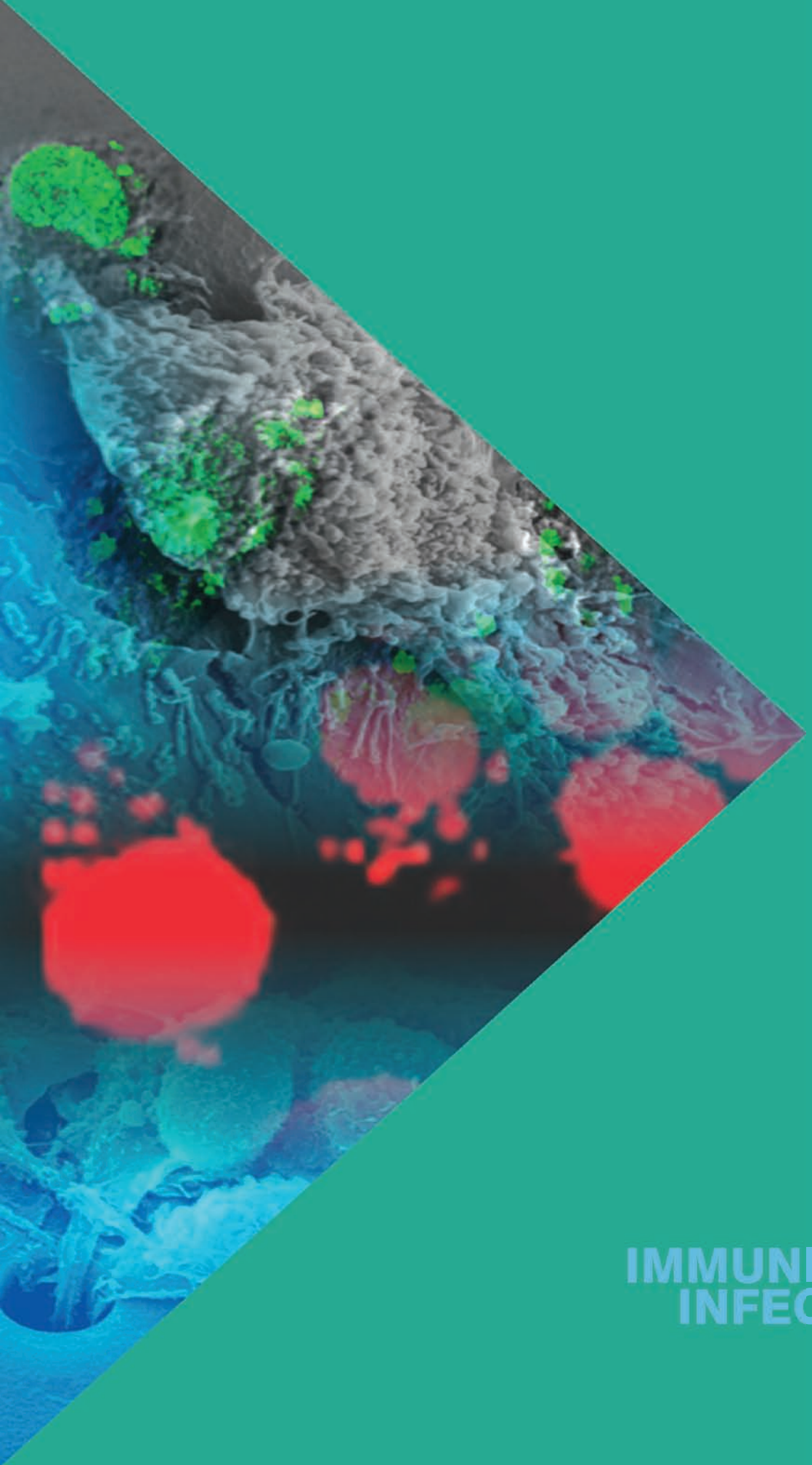
As NII attempts to meet the challenges in the coming year, my heartfelt thanks and congratulations to the staff of the institute because it would not have been possible to progress without their enthusiastic and inspiring participation. I am sure the existing staff members of the institute with their expertise and enthusiasm will further propel the institute forward and help it to sustain excellence to shine ahead and flourish in the coming years to contribute to the health care challenges of the future.

Dr. Chandrima Shaha
Director

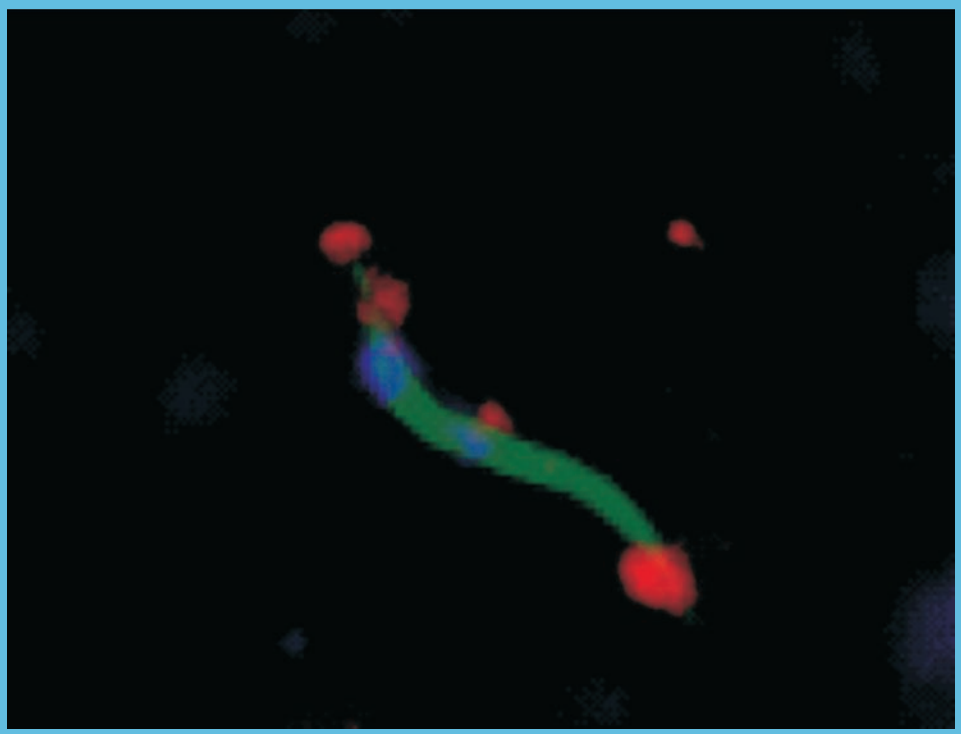
Date : September 23, 2016

RESEARCH REPORTS

IMMUNITY AND INFECTION



IMMUNITY AND INFECTION



Sporozoite staining with anti-PB-TIP polyclonal antibody shows protein secretion from the anterior side of the sporozoite (Arrow).

Satyajit Rath

Analysis of antigen processing and presentation

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During 2015-2016, substantial progress has been made in the ongoing analysis of the role of Bruton's tyrosine kinase (Btk) in immune system development and function.

The B cell lineage defect manifested in mouse Btk-deficiency (X-linked immunodeficiency; XID) is subtle. While XID mice show normal numbers of splenic transitional immature B cells, this peripheral maturation block is moved upstream in XID-TCRbeta-null, XID-CD40-null or XID-MHCII-null mice, who have near-normal numbers of early transitional cells but show substantial reduction of the late transitional stage. Thus, in the absence of functional Btk, CD4 T cells provide a part-redundant signal via CD40 to the T1-stage B cells in the periphery to regulate their developmental transition.

While the spleen is thought of as a major site for transitional B cell maturation, splenectomy had no effect on the numbers of mature peripheral B cells in Xid mice, indicating that the spleen is not a non-redundant site for T cell-dependent transitional stage maturation of Xid B cells. Also, while B cell trafficking and recirculation through peripheral lymphoid organs depends on interactions of adhesive molecules such as Icam-1, splenic B lineage cell numbers remained comparable between Xid and Xid+Icam1-null DM mice, ruling out a major role for Icam-1.

Btk is known to function downstream of the BCR and control the PI-3-kinase (PI3K)-Akt pathway, which is a major regulator of cellular metabolism, likely to be a significant factor in developmental transitions. Yet, neither cell size, nor mitochondrial mass, nor mitochondrial activity, nor uptake of a fluorescent glucose analog, nor levels of intracellular ROS, all metabolism-dependent parameters, were

different between WT and Xid transitional B cells, or between Xid and Xid+CD40-null transitional B cells. These data indicate that the metabolic status of peripheral B lineage cells may be largely independent of both Btk and CD40 signals.

We found that Xid transitional B cells were much more susceptible to death in culture than their WT counterparts. While both Baff and CD40 ligation brought about substantive and comparable rescue of WT transitional B cells, XID transitional B cells showed very poor rescue in response to Baff, yet CD40 ligation induced their rescue at levels comparable to that of WT cells. These data suggested that regulation of cell death may be a major issue. Roles for both the canonical and the non-canonical NF-kappa B pathways have been implicated in successful B cell maturation. We, therefore, analyzed these pathways and their significance for transitional B cells. CD40 ligation-mediated rescue from transitional B cell death was independent of non-canonical NF-kappaB signaling, since transitional B cells purified from non-canonical NF-kappaB p100-null mice could not be rescued by Baff treatment but were efficiently rescued by CD40 ligation.

Together, our data now show that CD40-mediated signals from CD4 T cells provide a mechanism of peripheral transitional B cell maturation independent of the non-canonical NF-kappaB pathway, and thus contribute to the understanding of the complexities of peripheral B cell maturation.

Publications

Original peer-reviewed articles

1. Rathore DK, Nair D, Raza S, Saini S, Singh R, Kumar A, Tripathi R, Ramji S, Batra A, Aggarwal KC, Chellani HK, Arya S, Bhatla N, Paul VK, Aggarwal R, Agarwal N, Mehta U, Sopory S, Natchu UC, Bhatnagar S, Bal V, Rath S, Wadhwa N (2015) Underweight full-term Indian neonates show differences in umbilical cord blood leukocyte phenotype: a cross-sectional study. *PLoS One* **10**: e0123589.
2. Verma S, Mohapatra G, Ahmad SM, Rana S, Jain S, Khalsa JK, Srikanth CV (2015) Salmonella engages host microRNAs to modulate SUMOylation: a new arsenal for intracellular survival. *Mol. Cell. Biol.* **35**: 2932-2946.

Prafullakumar B. Tailor

Understanding the role of Interferon regulatory factors in Dendritic Cell development and innate immunity

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TGF-β/BMP signaling has pleiotropic effects on immune cell development and functions. A recent study demonstrated that IRF8 directly controls *Itgb8* gene in APCs and triggers development of T_{reg} and Th_{17} cells by activating latent TGF-β. TGF-β treatment induces *Id2* gene expression in DCs. *Id2* expression is essential for classical CD8α$^+$ DC development and we recently reported that Irf8 expression leads to an increase in *Id2* transcript levels. These findings suggest that Irf8 may play an important role in controlling TGF-β signaling in DCs. Hence, to study the effect of Irf8 expression on TGF-β/BMP signaling, we performed our study using a TGF-β/BMP pathway focused PCR array based approach in DC9 (Irf8$^{-/-}$) cells. Our PCR array analysis identified *Acvrl1* gene (*Alk1*, a type I receptor of TGF-β superfamily), highly induced by *Irf8* in DC9 cells. To assess the significance of *Acvrl1* in DC biology, we examined its expression in different DC subtype populations from spleen which suggested that *Acvrl1* is specifically expressed in CD8α$^+$ DC subtype. To confirm the selectivity of *Acvrl1* gene expression in CD8α$^+$ DCs, we conducted PCR array analysis of BMDCs from mice carrying BXH-2 (*Irf8*^{R294C}) mutation in the Irf8 gene. Irf8 is essential for the development of pDCs and CD8α$^+$ DCs and mice homozygous for *Irf8*^{R294C} mutation showed selective block in the development of CD8α$^+$ DCs. The PCR array for BXH-2 mice confirmed that *Acvrl1* is indeed a CD8α$^+$ DC specific marker. We recently demonstrated that *Irf8* increases Batf3 and Id2 transcript expression and these transcription factors have a synergistic effect on Irf8 directed classical CD8α$^+$ DC development. Consistent with these findings co-expression of Batf3 and Id2 led to a synergistic increase in Irf8 induced Acvrl1 expression. This observation suggests that transcription factors essential for CD8α$^+$ DC development co-operate with Irf8 to induce high levels of Acvrl1 and may partly explain differential expression of Acvrl1 in CD8α$^+$ DC as

opposed to pDCs. Gene expression analysis of DC subtypes from different tissues showed selectively high levels of Acvrl1 in CD8α$^+$ DC (or its equivalent DC population) thus making it a good candidate for a CD8α$^+$ DC specific marker.

To understand the significance of ACVRL1 signaling in Irf8 directed DC development, we co-expressed Irf8 and Acvrl1 in DC9 cells. ACVRL1 signaling augmented Irf8 directed CD8α$^+$ DC development concomitant with the increase in subtype specific transcripts. Similarly, co-expression of Irf8 and Bmp9 (GDF2), one of the high affinity physiological ligand for ACVRL1 also synergistically increased Irf8 directed CD8α$^+$ DC development. These results suggested that BMP9-ACVRL1 signaling may play critical role in the selective development of CD8α$^+$ DCs (Figure 1).

Circulating BMP9 has been detected at a concentration of 2 - 12 ng/ml in human serum and it was demonstrated that ACVRL1-stimulating activity in serum is attributable to BMP9. Supplementation of FL-BMDC cultures with ACVRL1 high affinity ligand BMP9 (10ng/ml) led to a selective increase in CD8α$^+$ DC development and suppression of pDCs. Together, we demonstrate that ACVRL1 signaling contributes to DC diversity generation by specifically enhancing CD8α$^+$ DC development.

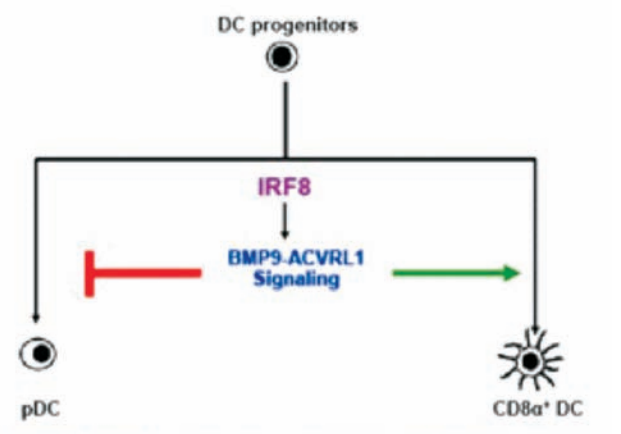

Figure 1: ACVRL1 signaling plays a critical role in CD8α+ DC development.

Vineeta Bal

Biology of T lymphocytes

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A. To study the effect of temperature on Th1/Th2 differentiation fate of CD4 T cells.

Since fever is a common outcome of an infection by various pathogens we are interested in understanding the immune pathophysiology associated with high temperature. We have begun addressing this question by examining the effect on T cell activation and differentiation at temperature upto 40°C *in vitro*. We observe that naïve CD4 (NCD4) T cells activated at 40°C produce more IL-4 and IL-13 on recall *in vitro*, with no or minimal change in interfeon-gamma (IFNg) producing capabilities, thus skewing cytokine balance towards Th2 effector phenotype. This is true not only for NCD4 cells isolated from C57BL.6 strain but also from BALB.c strain. NCD4 T cells isolated from healthy human adult donors, when activated *in vitro* show similar outcome as seen with mouse cells. While for a clear shift in cytokine readout activation at 40°C for 48 h seems necessary, for upregulation of GATA-3, a transcription factor responsible for differentiation towards Th2 effector phenotype, 6 h of incubation at 40°C is sufficient. Transient receptor potential vanilloid (TRPV) family of receptors which contribute to calcium transport has TRPV-1 as one receptor with Capsaicin as the agonist. TRPV1 is reported to sense increase in temperature as well. We observe that activation of NCD4 cells at 37°C in the presence of Capsaicin leads to Th2 effector

differentiation of the cell thereby identifying a potential signaling pathway which might be contributing to the temperature mediated differences in the outcome in T cell response *in vitro*.

B. To analyse the possible contribution of genetic and environmental components on immune cell phenotype in human adults.

We are in the process of collecting peripheral blood from healthy human adult volunteers to examine immune cell phenotype for about 25 different subsets. This is done to understand the contribution of genetic and/or environmental influence on the immune cytome in the absence of overt illness. For examining genetic influence we have collected blood from sibling pairs and our preliminary data on 30 pairs show that total B cell frequencies and B1 B cell frequencies between siblings and non-siblings were not different, whereas naïve CD4 cell frequencies, as well as total memory CD4 frequencies were different. These data suggest that niche for naïve and memory CD4 T cell frequencies may be genetically determined whereas it may not be the case with total B cells as well as B1 B cells. Data analysis is in progress.

C. To analyse the contribution of various immune components in dextran sulphate sodium (DSS) induced colitis.

Based on our earlier observations that expression of CD80 on non-hematopoetic cells, such as epithelial cells, may have a role in barrier function we have set up a colitis model in mice using daily feeding of 2.5% DSS. Our preliminary data show that as compared to wild type C57BL.6 mice fed with DSS CD80-null mice were relatively resistant to the development of inflammatory colitis.

Publication

Original peer-reviewed article

1. Rathore DK, Nair D, Raza S, Saini S, Singh R, Kumar A, Tripathi R, Ramji S, Batra A, Aggarwal KC, Chellani HK, Arya S, Bhatla N, Paul VK, Aggarwal R, Agarwal N, Mehta U, Sopory S, Natchu UC, Bhatnagar S, Bal V, Rath S, Wadhwa N (2015) Underweight full-term Indian neonates show differences in umbilical cord blood leukocyte phenotype: a cross-sectional study. *PLoS One* **10**: e0123589.

Agam Prasad Singh

Plasmodium proteins involved in virulence and host modulation: Host-Parasite interactions in *Plasmodium* Liver stages

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Theme of research

Plasmodium species introduce effector molecules into hepatocyte the cytosol to manipulate host metabolic and /or signaling pathways for its own benefit. Basic theme is to identify, new parasite molecules that affect the host cellular processes, and possible intervention strategies. The objective of this study is to identify new parasite derived proteins that are involved in host modulation, and required for parasites to grow and complete their life cycle. The current project aims to identify parasite proteins that play a role in liver stage parasite development. The parasite proteins can be evaluated for their vaccine potential, provided they could be expressed as recombinant proteins.

Protective T cell epitope in SLTRIP

Previously we have shown (Jaijayan DK et al., (2015) JBC 290: 19496-511) that SLTRIP immunization provides four log protection against sporozoite challenge. We have also shown that protection mainly comes from T cell response. Based on its vaccine potential we are now working towards the identification of minimal protective T cell epitope in the SLTRIP protein. SLTRIP protein is a 418 amino acid polypeptide and bioinformatics based analysis indicates a presence of multiple strong T cell epitopes. To know which of them is a protective a epitope, we have divided full-length protein into pieces (C2-C5) each ranging from 54-110 amino acids. Each of these pieces were sub-cloned in PGEX6P1 vector and expressed as GST fusion proteins in *E. coli* and purified using the GST affinity columns. Purified proteins were then used for immunization of mice and immunized mice were challenged with sporozoite to know the level of protection. Once the smaller protective region is identified, we will synthesize short and overlapping

peptides (~15-20 a.a.) and identify the minimal T cell protective epitope using these peptides in an antigen specific T-cell activation assay.

The role of host transcription factors in liver stage malaria parasite development.

By comparing the transcriptome of wild type or SLTRIP-KO parasite infected host cells, we identified four transcription factors that were heavily modulated in case of SLTRIP-KO infected host. We hypothesize that these transcription factors might play a strong role in parasite development. Accordingly, we have down modulated these transcription factors using the siRNA and tested how it affects the parasite growth. The siRNA were formulated into nano-particles that were capable of cell type specific delivery of the siRNA. Using the siRNA and *in vitro* culture system of infection with sporozoite, we found Mef2C, Sox10 and TCF4 knockdown decreases parasite growth by 96 ∓ 2%

The role of a putative immuno-modulatory protein PB-TIP in liver stage parasite development and host immune response.

Evidences are accumulating leading to state that the host immune response is modulated over the course of malaria. Using bioinformatics based analysis we have identified a putative T-cell modulatory protein from malaria parasites. So far we have expressed this protein in *E.coli* and raised polyclonal serum against the recombinant protein. Immuno-flourescence assay data confirmed its expression in Ookinete and Sporozoites but not in blood stage parasites. IFA images hints towards secretion of this protein from the anterior side of the sporozoite.

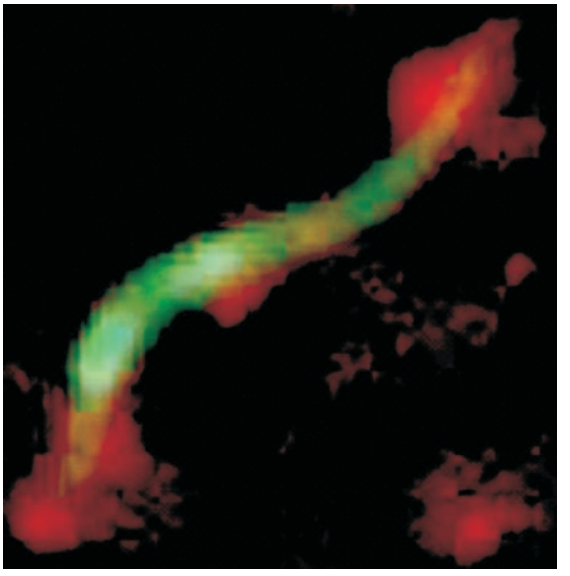

Fig. Sporozoite staining with anti-PB-TIP polyclonal antibody shows protein secretion from the anterior side of the sporozoite (Arrow).

Publications

Original peer-reviewed article

1. Jaijyan DK, Singh H, Singh AP * (2015) A Sporozoite and Liverstage Expressed Tryptophan Rich Protein Plays an Auxiliary Role in Plasmodium Liver Stage Development and is a Potential Vaccine Candidate. *J. Biol. Chem.* **290:** 19496-19511.

(* Corresponding author)

Review/Proceedings

1. Thakur R, Anand R, Tiwari S, Singh AP, Tiwary BN, Shankar J (2015) Cytokines induce effector T-helper cells during invasive aspergillosis; what we have learned about T-helper cells? *Front Microbiol* **6:** 429.

Akhil C. Banerjea

Genetic and functional analyses of host and HIV-1 genes that affect progression of HIV-1 and development of nucleic acid based antiviral approaches

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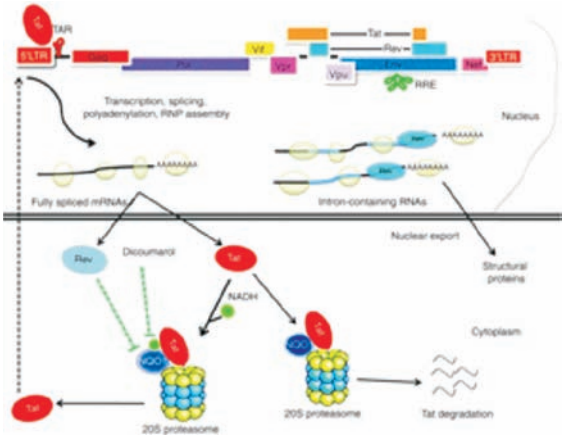
HIV-1 is known to evade host restriction factors for its own advantage and the virus tries to evade this restriction my multiple mechanisms. One such host factor is the DNA editing APOBEC3G. We characterized the Vif variants from North India and found novel mutations in several domains. Domains like phoshorylation were conserved and we observed that majority of our sequences were closely related to South African Vif C. We also came across some novel Vif B/C recombinants with different geographical location by phylogenetic analysis. Since the major activity of the Vif is the degradation of APOBEC proteins, we tested this ability with our variants and found that they differed greatly in this vital function. Intracellular regulation of HIV-1 Tat/Rev was studied and domains in Rev protein responsible for Tat degradation was identified. We also report that Dicoumarol, which is a potent inhibitor of NQO1, causes degradation of Tat protein and potently inhibits HIV-1 replication. NQO1 stabilized Tat protein in a dose-dependent manner but Rev degraded endogenous NQO1. Thus, Rev reduces the functions of Tat indirectly. Rev was also able to cause reduction in the ubiquitinated forms of Tat protein, earlier shown by others to be

important for its transcriptional activity which is measured by activation of HIV-1 LTR promoter activation. In a related study we observed that Tat stabilized MDM2 by phosphorylating Ser-166. The phosphomutant MDM2-S166A was fond to abrogate MDM2-mediated increase in HIV-1 replication. We observed that Vif interacted with AKTand functional implication is being explored. We also report that HIV-1 exploited cellular biosynthetic machinery by modulating MTORC for its own replication. The delicate balance between anabolism and catabolism is largely governed by MTORC. It is up regulated at 24 hours post infection but is drastically reduced at 48 hours. TRAF family of proteins are very important players in the various signalling pathways, like TLR, IL-R etc. They are poly functional and known to influence various biological activities like cell survival and cytokine production. We observed that TRAF3 and TRAF6 were upregulated post HIV-1 infection in human macrophages as well as by overexpression based studies. Functional implications of this observation is being explored, especially their kinetics since their (TRAF3 and TRAF6) activation has different outcomes. Ubiquitination and de-ubiquitination is a dynamic process and is ultimately responsible for the intracellular half life of any protein. We observed that HIV-1 Tat gets ubiquitinated intracellularly but remarkable stability was observed when USP7, a de-ubiquitinase (DUB), was used. This DUB was able to potently enhance HIV-1 replication by stabilizing Tat protein..



Mechanistic model of Rev- and dicoumarol-mediated downregulation of Tat and HIV-1 replication

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Anna George

Study of mucosal immune responses

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We have reported previously that CD40-signaling *in vivo* via homeostatic T cell-B cell interactions inhibits *Blimp-1* transcripts in naïve B cells. Over the past year, we have been dissecting the molecular basis of the repression. First, we tested for effects on mRNA stability, and actinomycin D experiments indicated no modulation of *Blimp-1*mRNA stability. Next, we determined whether CD40-signaling might induce miRNAs, and focused on miR-23b, miR-30a and miR-125b, which have been shown previously to silence Blimp-1 transcript. We compared expression of these miRNAs in naïve B cells from CD40$^{-/-}$ and TCR$\beta^{-/-}\delta^{-/-}$ mice with respect to their WT controls, and in WT B cells treated with anti-CD40 antibody for 24 hours. We found that *miR-125b* transcripts are significantly lower in *ex vivo* naïve B cells isolated from both CD40-CD40L interaction-deficient environments and that treatment of WT B cells with anti-CD40 antibody leads to an induction of this miRNA. No significant differences were seen in *miR-30a* levels, and *miR-23b* was variably modulated-it was lower in *ex vivo* B cells from CD40$^{-/-}$ and TCR$\beta^{-/-}\delta^{-/-}$mice, but was not significantly induced by CD40-ligation. To confirm the modulatory effects of *miR-125b* on *Blimp-1*, we transfected the M12.4.1 B cell line with *miR-125b* mimic or *miR-125b* inhibitor and assessed *Blimp-1* transcript abundance 24 hours later. We found that they reduced (and increased, respectively) *Blimp-1* transcript and protein abundance in the transfected cells. Transfection with miR-23b mimic and inhibitor showed more modest modulation.

In addition to its effect on repressing Blimp-1 transcripts, we found that CD40-signaling also reduces BLIMP-1 protein amounts by an independent mechanism involving induction of the E3 ubiquitin ligase Hrd1, which targets

BLIMP-1 for proteasomal degradation. It also inhibits the transient ER stress response induced within 24 hours of B cell activation and Hrd1 feeds into this pathway by inhibiting IREα (a core component of the unfolded protein response that accompanies ER stress). Treatment of WT B cells with the proteasomal inhibitor MG132 led to an increase in both IRE-1α and BLIMP-1 amounts, thus supporting the link between CD40-mediated induction of Hrd1 and lower amounts of the two proteins. Interestingly, transfection of M12.4.1 cells with the miR-125b inhibitor led to a decrease in Hrd1 transcript and protein, and transfection with the mimic led to an increase, indicating regulation of Hrd1 by miR-125b. Whether this modulation operates by repressing a repressor or by actively inducing transcription, as has been suggested for miR-37, is unclear.

Together, our data indicate that CD40-signaling induces *miR-125b* that targets *Blimp-1* transcripts, and increases amounts of the ubiquitin ligase Hrd1 that targets BLIMP-1 protein for proteasomal degradation. CD40-signaling also inhibits the early unfolded UPR of activated B cells that precedes the induction of terminal differentiation, and Hrd1 feeds into this pathway by targeting the core UPR component IRE1α. Thus, differentiation choices of naïve B cells may be influenced by cognate and bystander T-B interactions that occur prior to initiation of B cell proliferation.

Ayub Qadri

Analysis of *Salmonella Typhi*-host cell interaction

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Different *Salmonella* serovars produce different clinical manifestations depending upon the type of host. *Salmonella Typhi* causes systemic infection, typhoid, in humans while infection with non-typhoidal serovar *Salmonella Typhimurium* produces only self-limiting localized gastroenteritis. On the other hand, S.Typhi does not establish infection in mice whereas *S.Typhimurium* infection in susceptible strains of mice results in a systemic outcome that is analogous to human typhoid. The reasons for different clinical outcomes and for the host specificity exhibited by closely related *Salmonella* serovars have not been elucidated, and the strategies which pathogenic *Salmonella* employs to evade host immune responses in order to establish infection are not completely understood. Work in our laboratory addresses these and related aspects of host-pathogen interaction during infection with *Salmonella*.

Myeloid - derived suppressor cells modulate splenic T-cell responses during infection of mice with *Salmonella Typhimurium*

T cells and T cell-derived cytokines play a crucial role in immunity against microbial pathogens including *Salmonella.* We showed previously that the ability to secrete IL-2 in response to stimulation with anti-CD3 antibody is significantly reduced in splenic T cells following infection of mice with *Salmonella Typhimurium*. This reduction was specific to IL-2 as these T cells secreted higher amounts of IFN-γ and IL-17 when compared with T cells from uninfected mice. The ability of T cells to proliferate in response to TCR activation was also not affected by *Salmonella* infection. We now show that the inhibitory signal to downregulate IL-2 secretion is provided by $Gr1^{+}CD11b^{+}$ myeloid-derived suppressor cells (MDSCs) which are recruited to the spleen as *Salmonella* infection progresses. Experiments carried out with splenocytes from *Salmonella*-infected IFN-$\gamma^{-/-}$ and $iNOS^{-/-}$ mice reveal that this downregulation of IL-2 secretion from T cells might be produced through IFN-γ - dependent nitric oxide-mediated mechanism. These results suggest that MDSCs might be involved in modulating long-term immunity against infection with *Salmonella*.

Caspase-1 has a cell death independent role in *Salmonella* infection

We showed previously that treatment of splenic macrophages obtained from *S.Typhimurium* infected mice with caspase-1 inhibitor *ex vivo* significantly reduces intracellular bacterial load while treatment with caspase-1 activator increases the intracellular bacterial burden. To understand this phenomenon further, we carried out a comparative analysis of *Salmonella* infection in bone marrow - derived macrophage cell lines made from WT and caspase-1 deficient mice. As expected, caspase-1 expressing macrophages underwent frank cell death following infection with *S.Typhimurium* which wasn't readily seen in caspase-1 deficient macrophage line. Several hours post infection, WT macrophage cultures showed a lesser number of intracellular bacteria due to a presence of reduced number of live cells while caspase-1 deficient cultures had a higher number of intracellular bacteria due to more number of live macrophages. Interestingly, however, *Salmonella* derived from WT macrophages showed better growth in nutrient poor medium as compared to bacteria obtained from caspase-1 - deficient macrophages suggesting that cues derived from casapse-1 might have imprinted upon bacteria the capability to replicate better in the nutrient poor atmosphere reminiscent of the intracellular environment inside infected macrophages. Preliminary data shows that *Salmonella* isolated from WT macrophages have higher levels of ATP as compared to bacteria isolated from caspase-1 deficient macrophages. These findings reiterate a cell-death independent role for caspase-1 in the establishment of infection with pathogenic *Salmonella*.

Anti-*Salmonella* antibody response relevant to immunity is highly serovar specific

Salmonella Typhi and *Salmonella Typhimurium* share a very high degree of homology at the genome level. Yet, currently available *Salmonella Typhi* vaccine Ty21a offers only limited protection against closely related *Salmonella* serovars including *Salmonella Typhimurium.* To understand reasons for serovar-specific immunity, we injected mice with live *Salmonella Typhi* and analyzed antibodies at different time points. Antibodies from immunized mice showed cross-reactivity with *Salmonella Typhimurium* antigens including LPS in ELISA. However, while these antibodies readily bound live *S.Typhi* when analyzed by flow cytometry, these did not show any detectable interaction with live *S.Typhimurium*. Preliminary analysis suggests that serovar specific and shared O-antigenic determinants of LPS may be differentially accessible on the surface of live *Salmonella* due to which cross-reactive anti-LPS antibodies may not bind live *Salmonella*. These findings provide an explanation for serovar-specific immunity provided by anti-O antibodies.

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Original peer-reviewed articles

1. Akhade AS, Qadri A (2015) T-cell receptor activation of human CD4(+) T cells shifts the innate TLR response from CXCL8(hi) IFN-γ(null) to CXCL8(lo) IFN-γ(hi). *Eur J Immunol*. **45**:2628-2637.
2. Garg R, Akhade AS, Yadav J, Qadri A (2015) MyD88-dependent pro-inflammatory activity in Vi polysaccharide vaccine against typhoid promotes Ab switching to IgG. *Innate Immun* **21**:778-783.
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Devinder Sehgal

Microbial interface biology and associated host immune response

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The theme of research is to decipher how *Streptococcus pneumoniae* (pneumococcus) causes disease and what interventions can be made to stop this from happening. The research is focused on the pneumococcal products and strategies that allow the pathogen to avoid being destroyed by the mammalian immune system, and the types of immune responses that can circumvent these strategies and products. The main objectives are (a) identification and characterization of pneumococcal virulence factors that are or may be related to pathogenesis, (b) *h*ow these virulence factors interact with the immune system and host cell to alter its cellular and molecular processes, and (c) *e*valuating the vaccine potential of pneumococcal surface proteins.

Functional characterization of a novel DNase from *S. pneumoniae* secretome

Pneumococci has evolved diverse strategies to evade the host immune system. Neutrophils play a dominant role in clearing *S. Pneumonia* from the host. Neutrophils accomplish this by phagocytosing the bacterial pathogen or trapping and killing the bacteria in neutrophil extracellular traps (NETs). The dissemination of pathogens like *S. Pneumoniae* in the host is restricted once they get trapped in NETs. Previously, we had identified SPD_1788 as a potential candidate DNase from pneumococcal secretome. Recombinant SPD_1788 showed DNase activity in the absence of divalent cations however, the DNase activity was enhanced in their presence. SPD_1788 showed a pH optima of 9 and could digest DNA of different topologies. Recombinant SPD_1788 efficiently degraded NETs released from PMA activated human neutrophils. SPD_1788 lacks a signal peptide and is released by *S. pneumoniae* in the form of outer membrane vesicles (OMVs). Confocal microscopy demonstrated that NETs were degraded by pneumococcal secretome. Further, OMVs from wildtype pneumococci degraded NETs whereas

OMVs from pneumococci deficient in *SPD_1788* showed little, if any, degradation of NETs. Mice survival experiments with wildtype and *spd_1788* deficient strain suggest that SPD_1788 is required for full virulence.

CbpL is a novel pneumococcal cell surface protein that interferes with adhesion to and invasion of host cells

With the notion of gaining valuable insights into host-*S. pneumoniae* interaction, we mined the pneumococcal exome for proteins that are conserved across serotypes.One such conserved protein that showed up in our screen was CbpL. Bioinformatic analysis predicted CbpL to be putative cell surface localized choline binding protein. Choline binding proteins have been documented to serve as virulence factors and to contribute to pneumococcal pathogenesis. A study from other laboratory have shown that antibodies against CbpL conferred protection to mice when challenged with a lethal dose of *S. pneumoniae*. Recombinant CbpL protein was purified to > 95% purity. The presence of CbpL in the choline binding proteins eluted from pneumococcal cells was confirmed by immunoblotting. This data indicated that CbpL is a choline binding protein. To understand the role of CbpL in host-pathogen interaction we tested whether recombinant CbpL binds to host cells. Flow cytometry based analysis demonstrated that CbpL binds to lung epithelial and alveolar macrophage cell lines. CbpL deficient pneumococci adhered to and invaded lung epithelial and alveolar macrophage cell lines 3 times more efficiently than wildtype and genetically complemented pneumococci *in vitro.* The binding of wildtype pneumococci with host cells was enhanced in the presence of polyclonal sera against CbpL. Providing exogenous recombinant CbpL reduced the adhesion of CbpL deficient mutant to host cells. This data suggests that CbpL interferes with adhesion of pneumococci to host cells. Intraperitoneally infected mice cleared CbpL deficient pneumococci from the lungs, blood, spleen and peritoneum with faster kinetics than wildtype pneumococci. The recovery of CbpL deficient pneumococci was significantly lower than the wildtype strain when incubated in freshly isolated blood or peritoneal lavage. Mice challenged intraperitoneally with a lethal dose of wildtype pneumococci succumbed to death within 48 hours whereas all mice infected with CbpL deficient mutant survived, indicating that CbpL is important for virulence. Taken together, our data indicates that CbpL plays a significant role in resisting clearance by the host by interfering with adhesion to and invasion of host cells.

Publication

Original peer-reviewed article

1. Vashishta M, Khan N, Mehto S, Sehgal D*, Natarajan K (2015) Pneumococcal surface protein A (PspA) regulates Programmed death ligand 1 expression on dendritic cells in a Toll-like receptor 2 and calcium dependent manner. *PLoS One* **10**: e0133601.

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Amulya K. Panda

Studies on immune response from antigen loaded biodegradable polymer particles and protein refolding from inclusion bodies

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The main objective of the project is to improve the immunogenicity of antigens entrapped in biodegradable polymer particles. High-throughput refolding of inclusion body proteins into bioactive form is another objective of the research group. Research in the following areas are conducted in the laboratory to achieve the objectives:

1. Analysis of immune response from antigen loaded polymer particles and evaluation of adjuvant properties associated with polymeric particle formulation. Evaluation of memory antibody response from polymer particle based immunization.

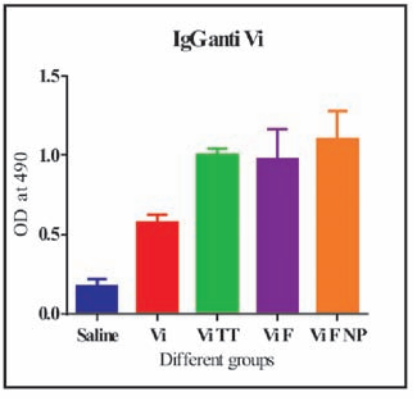
2. Solubilization and refolding of inclusion body proteins from *Escherichia coli*. This involves analysis of inclusion body formation during protein expression and understanding of protein aggregation with an aim to recover a higher amount of bioactive protein. A novel method of mild solubilization process is being developed in

the laboratory for high throughput recovery of bioactive protein from inclusion bodies.

(1) Development of a novel Vi polysaccharide based conjugate vaccine

Polymeric particles entrapping protein/carbohydrate antigens are being routinely used in the laboratory to improve their immunogenicity. It was observed that Vi polysaccharides entrapped in nanoparticles induce better antibody response than that observed with microparticles. As nanoparticle entrapped Vi polysaccharide was eliciting memory antibody response and promoting antibody isotype switching, it was of interest to compare the results with conjugate vaccine. Vi polysaccharide was conjugated to flagellin of *Salmonella typhi* using adipic acid dihydrazide linker chemistry. Conjugated and unconjugated protein and polysaccharide antigens were entrapped separately in PLA nanoparticles using double emulsion solvent evaporation method and used for immunization. It was observed that polymer nanoparticles antagonize the anti-inflammatory property of Vi polysaccharide antigen and promote pro-inflammatory cytokines which favours humoral immune responses. Further studies are underway to understand how polymer particle modulate the cytokine secretion pattern. The Vi-flagellin conjugate vaccine entrapped in polymeric nanoparticles elicited higher IgG type antibody repose from single dose immunization.

Anti Vi IgG antibody titers



Antibody estimation 2 weeks post immunization.

(2) Formulation and evaluation of pneumococcal vaccine using polymeric nanoparticles

It was observed that pneumococcal capsular polysaccharide entrapped in PLA nanoparticle provide protective immunity in mice model. Formulation studies are underway to entrap capsular polysaccharides of different *S. pneumoniae* serotypes. As serotype 14, 6A, 5 and 1 are the major virulent strains during pneumococcal infection, it was decided to evaluate the immunogenicity of these polysaccharides. Currently, we are formulating nanoparticles entrapping theses capsular polysaccharide to see if protective immunity can be achieved using polymeric delivery systems.

(3) Mild solubilization of inclusion body protein

Mild solubilization of inclusion body protein without using a high concentration of chaotropes results in high throughput recovery of bio active proteins. Attempts were made to screen suitable mild solubilization agent for efficient refolding of inclusion body proteins Among different organic solvents screened, trifluoroethanol (TFE) and butyric acid were found to be most potent solubilization agents and were able to solubilize a number of different inclusion bodies. Butyric acid could

not be studied for further studies as inclusion bodies solubilized in Butyric acid tend to aggregate upon refolding. Different biophysical approaches were used to find out the effects of TFE on the secondary and tertiary structure of human growth hormone. It was concluded that TFE acts as mild solubilization agent by stabilizing secondary structures and destabilizing tertiary structures.

Publications

Original peer-reviewed articles

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Review /Proceedings

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Patents

1. Meena J and Panda AK (2016) A novel typhoid vaccine comprise of conjugate of Vi polysaccharide with flagellin (Indian patent application 201611002743 filed on 25/01/2016
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Rahul Pal

Disorders of proliferation: Analysis of novel pathways and targets

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A. hCG and tumorigenesis

Studies in the lab have established that hCG mediates pro-proliferative effects on tumor cells, in addition to inducing the generation of inflammatory, angiogenic, pro-invasive and immunosuppressive mediators. hCG has also been shown to induce chemoresistance in tumor cells, and hCG in combination with TLR ligands induces synergistic increases in such chemoresistance. These observations are of significance since chemoresistance is frequently associated with poor prognosis in cancer patients. Whether endogenous TLR ligands, released upon tumor cell death subsequent to drug action, induce enhanced drug resistance (in combination with hCG) in the tumor cells that survive the initial drug assault is currently under investigation.

In mice implanted with syngeneic tumors, combination chemotherapy and anti-hCG immunotherapy induces significant decreased in tumor volumes and enhances animal survival. While the molecular basis of such synergy is being determined, the data provides support for the use of combination protocols in the therapy of gonadotropin-secreting tumors in humans that frequently acquire resistance to conventional therapy.

B. Systemic autoimmunity

The immunobiology of hemoglobin (Hb)

Investigations into the immunogenicity and antigenicity of Hb in the context of systemic autoimmunity are on-going. Higher concentrations of free Hb are observed in the plasma of lupus-prone mice than in the plasma of healthy mice, and splenocytes from aging, lupus-prone mice secrete significantly higher

levels of inflammatory cytokines when incubated with ferric Hb. Anti-Hb B cell precursor frequencies are also higher in lupus-prone mice, and circulating anti-Hb antibodies and kidney-adhered anti-Hb antibodies are readily detectable. Immunization of lupus-prone mice with Hb hastens the onset of glomerulonephritis. Hb thus demonstrates the attributes of an ideal autoantigen: It normally exists at a sequestered location, is inherently inflammatory, is both antigenic and immunogenic, and it triggers the spread of autoreactivity to lupus-associated antigens when injected to precipitate autoimmune pathology.

Characterization of apoptotic cell-reactive antibodies

The biological roles mediated by disease-specific IgM antibodies that specifically recognize apoptotic cells remain unclear in the context of systemic autoimmunity. Interestingly, such antibodies bind $CD11c^{+}$ bone marrow-derived dendritic cells (BMDCs) to a significant extent, stimulating the secretion of higher levels of inflammatory cytokines and mediating phenotypic maturation. Immunization of such antibodies in lupus-prone mice induces increases in total serum IgG levels and enhances anti-self reactivity. Apoptotic cell-reactive IgM antibodies, generally believed to mediate an anti-inflammatory role, may therefore, be potentially capable of influencing the course of the systemic autoimmune disease by affecting both innate and adaptive immunity.

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Rajni Rani

Study of genetic and immune factors associated with autoimmune disorders: Type1 Diabetes and vitiligo

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The project aims to decipher the Immunogenetic and autoimmune factors involved in the destruction of pancreatic beta cells and melanocytes in Type 1 diabetes (T1D) and vitiligo respectively. We aim to device ways to inhibit autoimmune responses in T1D.

Vitiligo is a multifactorial disease etiology of which is not precisely understood. While several hypotheses have been proposed including autoimmunity, it is not clear how the pigment producing melanocytes are destroyed by the autoimmune responses. So, the theme of this project is to understand the aetiopathogenesis of vitiligo with an aim to develop therapeutic approaches for the disease.

Type 1 diabetes

I superannuated from NII and was there only until the end of April 2015, however, my student Anshu Sharma is continuing her Ph.D. We wanted to understand whether we need to differentiate MSCs into insulin producing cells or precursors to insulin producing cells should be used to prevent an onset of Type 1 diabetes in NOD mice. MSCs grown in culture for a long time i.e. 11 to 13 passages showed expression of pancreatic lineage genes at mRNA level and also PDX1 protein using immunofluorescence. When these cells were given to NOD mice at 9th week, they gave equal protection from diabetes as insulin producing cells i.e. about 80% of mice were protected from T1D when treated with either late passage MSCs of differentiated insulin producing cells. These results have the potential to be translated in human type 1 diabetes scenario.

Vitiligo

We carried out a genome-wide array for the expression of micro RNAs in lesional (vitilliginous) and non-lesional (normal) skins of 18 patients for differential expression of 328 micro RNAs using the FlexmiR platform (Luminex). 28 microRNAs were found to be regulated. To study the role of miRNAs, potential targets for the upregulated miRNAs were obtained from 2 databases- Miranda and RNA Hybrid using the program GomiR. The micro-array data of keratinocytes transfected with miRNAs of interest were analyzed using DAVID. However, since not many genes were getting consistently regulated at mRNA level, it is possible that the miRNAs are affecting only the translational levels of the protein and not the transcript levels. To verify this, transfections were done to obtain native proteins and differential expression of proteins is being analyzed by another student of mine Utpreksha Vaish using MALDI-TOF analysis. Expression of target proteins regulated by different miRNAs will be checked for their role in the aetiopathogenesis of vitiligo.

Publications

Original peer-reviewed articles

1. Satchidanandam V, Kumar N, Biswas S, Jumani R, Jain C, Rani R, Aggarwal B, Singh J, Kotnur MR, Challu V, Chadha VK, Kumar P, Sridharan A (2016) Rv3881c from *Mycobacterium tuberculosis* elicits poly-functional $CD8^+$ T cells in PPD-positive healthy volunteers and affords significant protection in the guinea pig model. *J Immunol Tech Infect Dis.* **5**:2.
2. Satchidanandam V, Kumar N, Biswas S, Jumani R, Jain C, Rani R, Aggarwal B, Singh J, Kotnur MR, Sridharan A (2016) The secreted protein Rv1860 of *Mycobacterium tuberculosis* stimulates human polyfuctional CD8+ T cells. *Clin Vaccine Immunol* **23**:282-293.
3. Verma G, Varkhande SR, Kar HK, Rani R (2015) Evaluation of repigmentation with Cultured Melanocytes transplantation (CMT) compared to non-cultured epidermal cells (NCES) transplantation in vitiligo at 12th week reveals better repigmentation with CMT. *J Invest Dermatol* **135**:2533-2535.

Sangeeta Bhaskar

Study of immunotherapeutic potential of *Mycobacterium indicus pranii* (*MIP*) and the underlying mechanisms in animal models of tuberculosis and tumor model

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To investigate the protective efficacy of MIP immunization in live or killed form, through parenteral route / by the aerosol route, against subsequent infection with *M.tuberculosis* in animal models. Study of the immune response to *M.tb* in an animals immunized with MIP as compared to those immunized with BCG.

Evaluation of immunotherapeutic efficacy of MIP along with chemotherapy in animal model of tuberculosis.

To evaluate Immunoprophylactic and Immunotherapeutic activity of MIP in mouse syngeneic tumor model. Study of MIP as an adjunct to chemotherapy in combination with commercial anti cancer drugs in tumor bearing mice and simultaneous study of the mechanism of MIP mediated host immune activation.

A. Efficacy of MIP as a booster to BCG

BCG is effective against the severe form of childhood tuberculosis, however, its protective efficacy wanes and protection from TB in young adults is still a challenge, which indicates the need to boost the protective immune response of BCG. However, paradoxically, multiple doses of BCG do not give higher protection. Booster dose of MIP by both routes (Aerosol/s.c route) was given 2 months after BCG priming. Guinea pigs of 'control', 'only BCG' immunized group and 'BCG-MIP booster' groups were challenged with *M.tb*. Bacterial load in lungs and spleens were determined.There was 1 log reduction in lung bacterial load in BCG immunized animals as compared to control unimmunised group while BCG primed and MIP boosted group showed further 1 log reduction as compared to only BCG immunised group. Histopathological analysis confirmed the bacteriological findings. Significantly lower Gross pathological score was observed in the groups given booster immunization. Immune response in the lungs was studied after infection by analyzing the mRNA expression of cytokine genes. Significantly high expression of IL-12 and IFN- γ

was observed in the 'BCG-MIP booster' groups as compared to 'only BCG' group. IL-2 expression was moderate but it was up-regulated in 'BCG-MIP booster' group while expression of IL-17 was significantly high in the lungs of BCG-MIP (aerosol) group. TNF-α expression remained same across the groups. Expression of anti-inflammatory cytokines like IL-10, TGF-β was found to be similar in all the immunized groups.

Further, we evaluated the quality of T cell immune responses by measuring the cytokine production by CD4 T cells on a per-cell basis at 6 weeks post infection. BCG-MIP booster immunization in mice resulted in the heightened frequency of *M.tb* specific multi-functional CD4 T cells (3+) simultaneously producing IFN- γ, TNF-α and IL-2 in the lungs of infected mice as compared to 'only BCG' immunized mice, which correlated with higher protection observed in BCG-MIP boosted mice.

B. MIP enhanced the therapeutic efficacy of cyclophosphamide(CTX)

Chemo-immunotherapy, which leads to a two-sided assault on the malignant cells with cytotoxic drugs and anti tumor immune response, is a promising approach for the treatment of cancer. MIP, when given in a combined chemo-immunotherapy approach resulted in higher tumor regression as compared to chemotherapy group. Tumors in 'MIP+CTX' treated mice were infiltrated with higher levels of $CD4^+$ and $CD8^+$ T cells and $CD40^+$ and $CD80^+$ DCs and macrophages. 'MIP+CTX' treated mice had strikingly different tumor-microenvironment, compared with PBS, MIP or CTX treated mice. Tumor micro-environment in MIP treated mice was dominated by IFN- γ, whereas in 'MIP+CTX' treated mice, type I IFNs were expressed at significantly high levels. The higher cytolytic activity of $CD8^+$ T cells from 'MIP+CTX'treated mice, compared with CTX-treated mice was also observed. These results provide a valuable insight into the immune mechanisms leading to the higher antitumor efficacy of CTX used along with MIP.

C. Role of MIP in modulation of cell death pathways in *M.tb* infected macrophages

MIP immunotherapy by aerosol route when given along with drugs in animal models of TB, resulted in significant reduction of persistent bacteria as compared to 'only drug' group, which is an important observation from a clinical point of view. Antigen presenting cells at the primary site of *M.tb* infection uses several mechanisms for bacterial clearance including induction of programmed cell death. *M.tb* interferes with these host mechanisms at several levels. It inhibits phago-lysosomal fusion as well as apoptosis of infected cells. Effect of MIP on an autophagic process in macrophages and its role in phagosome maturation and phago-lysosome fusion in *M.tb* infected macrophages are being studied. theLevel of LC3II, which marks the increase in the autophagosome formation was found to be significantly high in 'MIP treated *M.tb* infected macrophages' as compared to 'only *M.tb* infected' macrophages. The role of MIP mediated autophagy in controlling the *M.tb* infection will be examined.

Publications

Original peer-reviewed articles

1. Kumar P, Bhaskar S* (2016) Analysis of T Cell proliferating and polarizing potential of murine dendritic cells in allogeneic-mixed leukocyte reaction. *Bio-protocol* **6**: e1750.
2. Kumar P, Marathe S, Bhaskar S* (2016) Isolation of genomic DNA from mycobacterium species. *Bio-protocol* **6**: e1751.

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Soumen Basak

Fine tuning of NF-kappaB Signaling

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Biochemical and genetic studies have orchestrated molecular connectivities between apparently "insulated" signal transduction pathways. Yet, a role of such interconnectedness in regulating stimulus responsive behavior of the cell system remains unclear. In an integrative approach, which combines experimental and mathematical studies, we have examined physiological and patho-physiological consequences of interdependent regulations of cell signaling pathways. In our ongoing research program, we have established the physiological role of network interactions *via* the NFκB system in fine-tuning inflammatory immune response to gut pathogen *Citrobacter rodentium*. We have also delineated the pathophysiological consequence of altered NFκB network circuitry in multiple myeloma in exacerbating environmental drug-resistance.Future studies will further address the role of crosstalk in the context the of physiology of immune response in lymphoid tissues.

Publications

Original peer-reviewed articles

1. Banoth B, Vijayaragavan B, Chatterjee C, Prasad MVR, Roy P, Basak S* (2015) Developmental LTβR reinforces TLR4 triggered inflammatory RelA/NF-kappaB responses to ameliorate the pathogen clearance in the gut. *eLIFE* **4**:e 05648.
2. Tsui R, Kearns J, Lynch C, Vu D, Ngo K, Basak S, Ghosh G, Hoffmann A (2015) The Rel-NFκB dimer generation module: monomer competition and a function for IκBβ. *Nat Commun* **6**:7068.

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Amitabha Mukhopadhyay

Modulation of intracellular trafficking in host cells by various intracellular pathogens

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We have shown earlier that *Salmonella* secretory protein, SipA binds with Syntaxin 8. In order to determine the role of SipA interaction with Syntaxin 8 in host cells, we have made a dual tag SipA construct in which 3XFLAG was placed just before DEVD motif and a 3XHA was placed at the C-terminal end of the protein ($SipA^{1-431}$-3XFLAG-DEVD-$SipA^{436-685}$-3XHA). This construct was episomally expressed in *Salmonella* (*Salmonella*:pdualtagSipA). Our results have shown that infection of HeLa cells with *Salmonella*:pdualtagSipA secretes this fusion protein in the cytosol and generates two fragments $SipA^{1-431}$-3XFLAG and $SipA^{436-685}$-3XHA. Interestingly, we have found that $SipA^{1-431}$-3XFLAG is colocalized with endogenous Syntaxin8 in the host cells an using anti-FLAG antibody, whereas, $SipA^{436-685}$-3XHA is colocalized with phalloidin labelled actin using anti-HA antibody. Thus, our results unequivocally demonstrated that SipA is secreted into host cell cytosol which is further cleaved into two fragments in DEVD motif and subsequently, N-terminal of SipA localized on Syntain8 containing vesicles. Finally, our preliminary results have shown that fusion between *Salmonella*:SipAKO bacteria containing phagosome with early endosomes is significantly inhibited in comparison to *Salmonella*:WT containing phagosome. Furthermore the addition of anti-syntaxin 8 antibody in the fusion assay also significantly inhibits the fusion between *Salmonella*:WT-containing phagosomes with endosomes. Taken together, these results indicate that *Salmonella* recruits Syntaxin8 on the phagosome by N-terminal of SipA and promotes fusion with early endosome possibly to inhibit their transport to the lysosomes.

We have also reported earlier that *Leishmania* infection in macrophages triggers the expression of Rab5 in the host cells. In the

reporting period, we have tried to understand the significance of Rab5a overproduction in the host cells by *Leishmania* infection. Our results have shown that *Leishmania* containing phagosomes recruit and retain Rab5a and EEA1 even after 48 hrs of infection indicating that *Leishmania* resides in the early endosomal compartment. However, we have also found that *Leishmania* containing phagosomes also recruit LAMP1 and cathepsin D on their phagosomes as observed previously. It could be possible if *Leishmania* infection somehow blocks the processing and trafficking of lysosomal enzymes in the early endosomes. Therefore, we have over expressed Rab5a:WT, Rab5a:Q79L or Rab5a: S34N protein in the HeLa cells and determined the trafficking of lysosomal enzymes. Interestingly, we have found that over expression of Rab5a:WT and Rab5a:Q79L blocks the trafficking of lysosomal enzymes in the Rab5 positive early endosomal compartment.

Taken together our results indicated that *Leishmania* overproduces Rab5a and thereby blocks the trafficking of lysosomal enzymes in the early endosomes. These results are further supported by the fact that size of the cathepsin D in the *Leishmania* infected macrophages are found to be predominantly about 52-48 kDa which is the size of procathepsin. Thus, our results have shown that *Leishmania* resides in the early endocytic compartment and also blocks the processing of lysosomal enzymes to survive in the host cells

Publication

Original peer-reviewed article

1. Bahl S, Parashar S, Malhotra H, Raje M, Mukhopadhyay A (2015) Functional characterization of monomeric GTPase Rab1 in the secretory pathway of Leishmania. *J. Biol. Chem.* **290**:29993-30005.

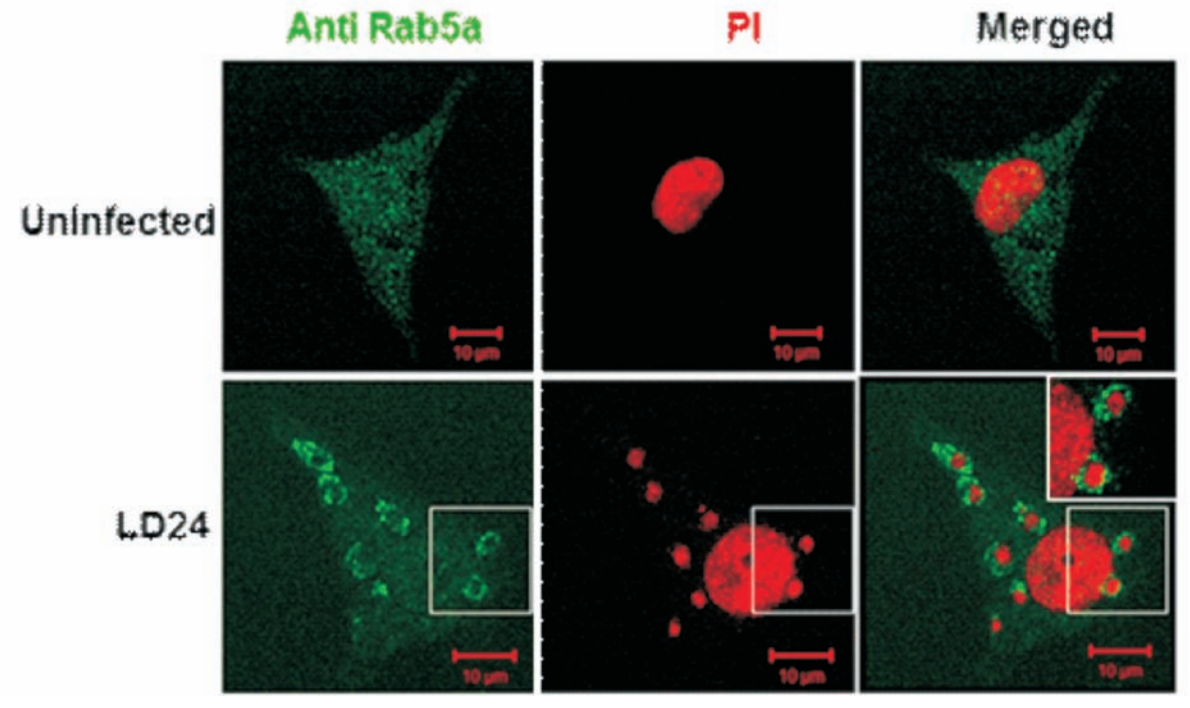

Fig. *Leishmania* recruits rab5a on its phagosomes in macrophages.

Nimesh Gupta

Mechanism of humoral immunity development and protection to Japanese Encephalitis vaccine

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The ultimate aim of our laboratory is to identify the approaches to improve vaccination strategies, principally, by harnessing the follicular T helper (TFH) cells. To understand the differentiation and function of TFH-cells and to identify the mechanism of their regulation during the germinal center reaction, we employ human vaccines as a model. To advance our efforts into translational research the laboratory is determined to work at the interface of basic and clinical research. Following is our recently ongoing project, which is under consideration for extramural funding by Department of Biotechnology.

Project Description

Live attenuated JE-vaccine has a key problem of short effector memory leading to waned immunity in 2-3 years. Although antibodies serves as correlate-of-protection the cellular component formulating long-lived humoral immunity is not known. Follicular T helper (TFH) cells are the specialized subset providing help to B-cells and constituting the germinal center derived long-lived antibody protection. Due to its sub-optimal efficacy and likelihood of immediate translation, JE-vaccine becomes an interesting model to investigate TFH-cell biology. Hence, the vital theme of the proposal is to illuminate the differentiation, function and regulation of TFH cells in JE-vaccination. Factors responsible for optimal TFH-cell responses will be identified using cutting edge approaches in antigen-specific manner. Moreover, the repercussion of TFH-cell modulation on long-lived humoral response and broad protective efficacy will be explored in a novel challenge model of JE.

Hypothesis

The main hypothesis is that the TFH cells are indispensable for JE-vaccine induced long-term humoral immunity. Understanding TFH-cell differentiation in sufficient detail will allow the

optimization of TFH-cell responses to maximize the development of protective antibodies, and the development of long-term humoral immunity in JE-vaccination.

Key Questions

Studies in the framework of this proposal are aimed at addressing following questions:

(i) Identifying the TFH-cell intrinsic factors that could be modulated to boost TFH-cell responses to JE-vaccine.

(ii) To identify the mechanism of TFH-cell regulation by follicular Tregs and to exploit the resulting unleashed TFH-signature in improving the vaccine efficacy.

(iii) To evaluate FcRn-deficient mice as a novel challenge model for JE-vaccine efficacy studies.

The Outcome of Proposed Study

The framework of the project is fundamental research and thus, the establishment of this program will certainly add to the strength in vaccine research domain. The outcome of proposed research has an enormous socio-economical impact for JEV endemic countries like India.

The studies that are in the framework of proposed research may ultimately provide:

1. The unique immune signature of commonly used JE-vaccine.
2. Knowledge on early immune correlates of protective immunity to JE-vaccine.
3. The regimen that can boost TFH cells leading to augmented antibody responses.
4. A new pre-clinical model for evaluation of the long-term efficacy of a vaccine.
5. The Basis for future studies investigating T-cell adjuvant or designing a novel vaccine.
6. The appealing lead for prospective clinical studies in JE-endemic countries.

Publications

Original peer-reviewed articles

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2. Ing M, Gupta N, Teyssandier M, Maillère B, Pallardy M, Delignat S, Lacroix-Desmazes S (2016) Immunogenicity of long-lasting recombinant factor VIII products. *Cell Immunol* **301**:40-48.

REPRODUCTION AND DEVELOPMENT

REPRODUCTION AND DEVELOPEMENT

Subeer S. Majumdar

Studies of Sertoli cells and spermatogonial stem cells of the testis and other endocrinology related research

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We have developed series of techniques for making transgenic mice and rat using testicular germ cells for gene integration and propagation and constantly working to refine such techniques further abolishing need to kill animals and reducing the use of animals. Through these indigenous methods of transgenesis, we have generated several animal models of different biomedical conditions and served to other research labs of the country. We also work towards analyzing differential gene expression by testicular Sertoli cells (Sc) obtained from spermatogenically active vs. inactive testes to identify Sc factors regulating germ cell division and differentiation with an intent to divulge unknown (inborn or environmentally induced) non-hormonal causes of idiopathic male infertility and genes responsible for age specific production of important testicular factors required to generate quantitatively and qualitatively normal sperm. Tetraspanin 8 (Tspan 8), Wnt3,

DKK3, Sostdc1 and Nor1 have already been identified by us as differentially expressed Sc genes regulating spermatogenesis. Transgenic animals overexpressing or suppressing the expression of such genes are generated and used by us to evaluate their functions. A study was designed to find out differential levels of miRNA expressed in immature and mature Sertoli cells and to understand how they might affect regulation of spermatogenesis using a microRNA species. For this purpose, total RNA was isolated from cultured infant and pubertal Sertoli cells and small RNA fractions were sequenced on an illumina platform. Sequencing analysis revealed 128 microRNAs to be up regulated and 288 microRNAs to be down regulated in pubertal Sertoli cells in comparison to infant Sertoli cells. Some of these microRNA were validated using separate sets of Sertoli cell culture and found to be truly differentially expressed in infant and pubertal Sertoli cells. We found that several conserved microRNA were differentially expressed. Bioinformatic analysis using DIANA miRNA analysis pipeline revealed that the microRNA Mir-504-5p, mir-878-3p, mir-125a which target important signaling pathways such as Wnt signaling and cancer related pathways were augmented in Sertoli cells during puberty. Since a single microRNA can target many of genes we picked up one of the differentially expressed microRNA mir-504-5p and found its important role in regulating spermatogenesis using transgenic mice. We are also working with other approaches for identifying such differentially regulated genes, like multiomics approach exploiting genome, transcriptome, and proteome.

Along with this, we are working towards a generation of transgenic (genetically modified) and non-transgenic (non-genetically modified, nonGMO) bioreactors for the production of human therapeutic protein (medicine) in milk to make them cheaper and affordable for masses. As an alternative approach, we are trying to transfect mammary epithelial cells directly *in vivo.* Transgenes delivered in this way interact with cell and integrate into the genome at the somatic level causing genomic alteration of somatic cells. This does not genetically alter the crucial germ line of the species, hence avoiding issues related to genetically modified organisms (GMO) involving germ-line gene integration. We were successful in transfecting mammary epithelial cells by direct injection of transgene entrapped virosomes in udder glands of mice and in expressing human interferon gama (hIFN- γ) in the milk the (~6.6ng/ml of milk) of mice. The study will be extrapolated in farmed animals.

Publications

Original peer-reviewed articles

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5. Sarkar H, Arya S, Rai U, Majumdar SS (2016) A study of differential expression of testicular genes in various reproductive phases of *Hemidactylus flaviviridis* (wall lizard) to derive their association with onset of spermatogenesis and its relevance to mammals. *PloS One* **11**:e0151150**.**
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Anil Suri

Cellular and molecular biology of human cancer

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Colorectal cancer (CRC) is the third most common cancer worldwide and accounts for 8% of total cancer related deaths. Moreover, the prognosis of CRC is poor, with 5-year survival rate of only 50% (15-65%). Hence, there is a need of a target molecule for early detection and diagnosis of CRC for better cancer management. In the present study, we investigated the expression of A-kinase anchor protein 4 (AKAP4), a new member of cancer testis antigen in various clinical stages and histopathological grades of CRC tumors and evaluated the humoral response in these patients. In addition, we also examined the AKAP4 gene and protein expression in eight colon cancer cells (CACO-2, COLO205, COLO320, HCT15, HCT116, HT-29, SW480, SW620) and normal colon epithelial cell and tissue lysate. Further, to study the effect of ablation of AKAP4 on tumorogenic properties at a molecular level in COLO205 and HCT116 cells, gene silencing approach was employed which showed the reduction in cellular proliferation and apoptosis *in vitro* and *in vivo*

xenograft mouse model. Our data suggests that AKAP4 is expressed in early stages and thus could be used as a biomarker for early diagnosis and potential therapeutic target for the development of better CRC treatment management.

Our results revealed *AKAP4* gene expression in CaCo-2, COLO205, COLO320 DM, HCT-15, HT-29, SW480 and SW620 colon cancer cell lines by quantitative real-time PCR which was further validated by western blotting and flow cytometric analysis for AKAP4 protein expression. We also probed AKAP4 protein in COLO205 and HCT116 which revealed predominantly cytoplasmic localization and co-localized in various organelles. RT-PCR and IHC studies results revealed AKAP4 transcript and protein in CRC specimens. We observed 84% of CRC specimens were positive for AKAP4 transcript and protein. We further analyzed our data that revealed AKAP4 transcript and protein expression in 88% (7/8) stage I, 84% (31/37) stage II, 88% (92/104) stage III and 74% (38/51) stage IV patients whereas no AKAP4 transcript and protein expression was detected in ANCT or normal colon (Figure 1).

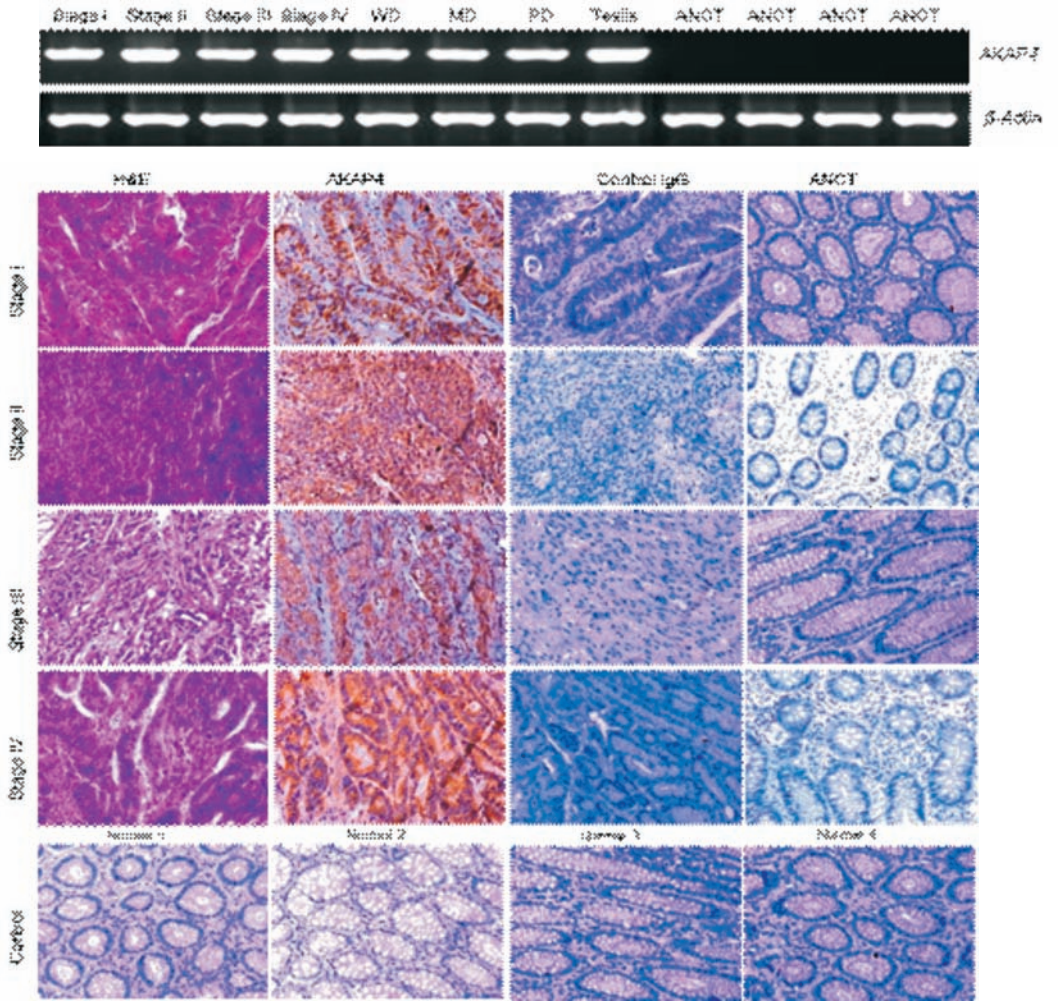

Figure 1. AKAP4 gene and protein expression in various stages of colon cancer specimens.

Gene silencing using shRNA target against *AKAP4* gene was used to study the role of AKAP4 in various malignant properties in both COLO205 and HCT116 cells in all *in vitro* assays. COLO205 were used to study *in vivo* xenograft mouse model. Knockdown of AKAP4 inhibited cellular growth and colony forming ability of COLO205 and of HCT116 cells. Ablation of AKAP4 also resulted in apoptosis of both COLO205 and HCT116 cells as shown in scanning electron microscope (SEM, Figure 2A). Subsequently, various anti-apoptotic and pro-apoptotic molecules were also investigated in both AKAP4 shRAN3 and NC shRNA treated cells. Our results revealed that pro-apoptotic molecules (AIF, APAF1, BAD, BID, BAK, BAX, PARP1, NOXA, PUMA and CYC-C and caspase proteins Caspase 3, Caspase 7, Caspase 8 and Caspase 9) were up regulated. However, anti-apoptotic molecules (BCL2, BCL-xl, CIAP2, XIAP, Axin2 and Survivin) were down regulated. Ablation of AKAP4 protein resulted in the reduction in migration and invasive abilities of COLO205 and HCT116 cells. Further studies on various signaling pathways contributing towards migration and invasion of cells revealed that expression of epithelial marker E-cadherin was up regulated, while N-cadherin, P-cadherin, SLUG, α SMA, SNAIL, TWIST, and Vimentin, were down regulated. Invasion molecules matrix metalloproteinase (MMP) like MMP2, MMP3 and MMP9 were also down regulated with AKAP4 ablation (Figure 2B).

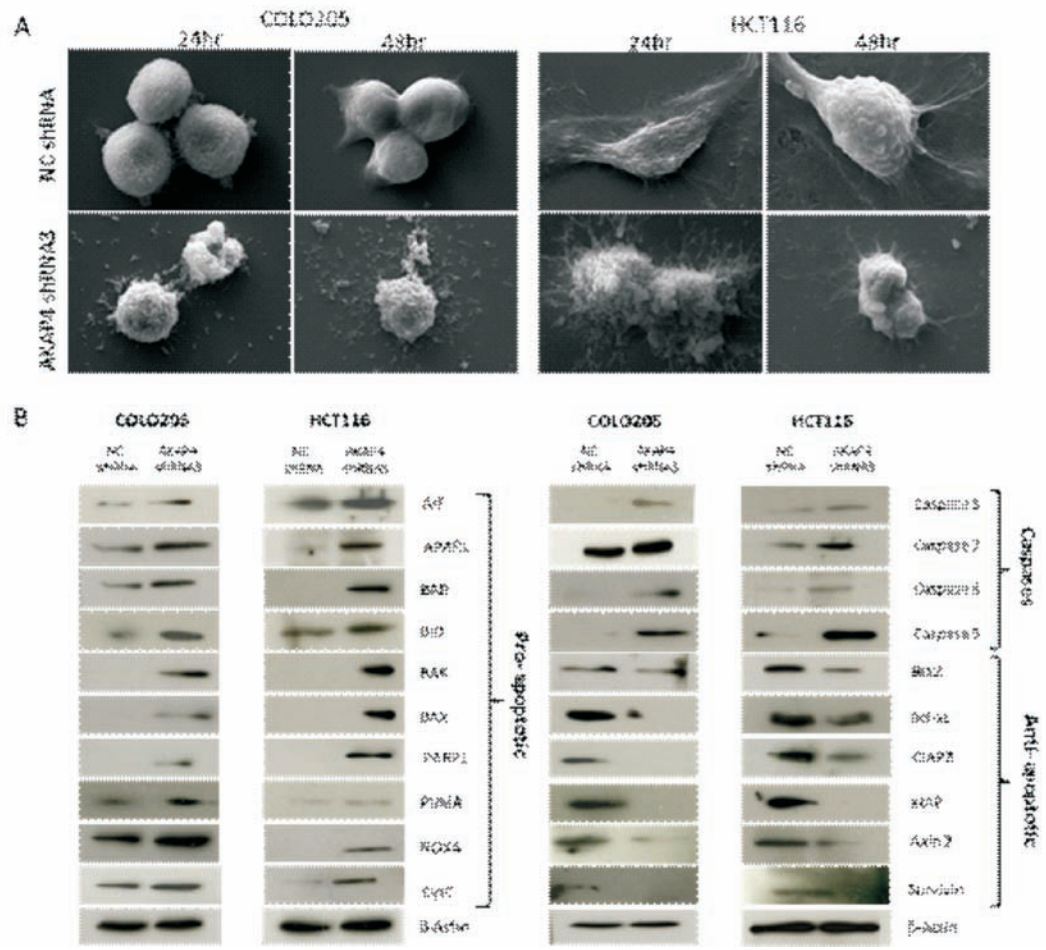

Figure 2) The scanning electron microscope (SEM) images of COLO 205 and HCT 116 cells transfected with AKAP4 shRNA3 or NC shRNA. **B)** The Western blots show changes in the expression of various molecules that are involved in apoptotic pathway in COLO 205 and HCT 116 cells when transfected with AKAP4 shRNA3 or NC shRNA.

To validate our *in-vitro* findings we investigated the role of AKAP4 in *in-vivo* colorectal xenograft mouse model. Our studies showed a significant decrease ($p<0.0001$) in tumor growth and Western blot analysis and IHC revealed the down regulation of AKAP4 and PCNA protein in AKAP4 shRNA3 treated mice as compared to NC shRNA treated .

IHC studies also demonstrated the down regulation of cellular proliferation molecules and up regulation of p16 and p21 in xenograft tumor tissues. We also found up regulation of proapoptotic and down regulation of anti-apoptotic molecules in animals treated with AKAP4 shRNA3 target. Validation of EMT molecules in xenograft tissues sections also revealed down regulation of αSMA, Vimentin, TWIST, SNAIL, SLUG, N-cadherin along with invasion molecules MMP2 and MMP9, whereas expression of epithelial marker E-cadherin was upregulated.

In conclusion, AKAP4 is a novel cancer testis antigen and may have a potential for developing as a new clinical therapeutic target for CRC treatment

Publications

Original peer-reviewed articles

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Review/Proceedings

1. Suri A*, Jagadish N, Saini S Gupta N (2015) Targeting Cancer testis antigens for biomarkers and immunotherapy in colorectal cancer: current status and challenges. *World J Gastroint Oncol.* **7**: 492-502.

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Asok Mukhopadhyay

Study on expansion and plasticity of bone marrow stem cells

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In past one year our laboratory worked on following three areas: (a) liver regeneration by bone marrow-derived cells and study of the mechanism for the reversal of liver fibrosis and A1ATD, (b) trans differentiation of MSCs to neuronal precursors, and (c) stem-ness in tumor cells and cancer progression.

A. Plasticity in BM cells

The recipient SCID-PiZ mice sacrificed after 3 months of transplantation of hMSCs. Transplantation of cells resulted in significant reduction of PAS-stained globules in hepatocytes. The donor-derived cells were changed into hepatic phenotype as they expressed albumin and CK18 antigens. Gene expression analysis revealed that many human genes such as human *TAT*, *CYP2B6* and *GYS1*, attributed to liver functions, were expressed in the recipient's liver. The differentiated hepatocytes also expressed alpha-1-antitrypsin protein.

B. Trans differentiation of human Wharton Jelly (WJ) MSCs into neural precursors

The canonical β-catenin pathway was found to be constitutively active in human WJ-MSCs. Thus by the combined effect of FGF2 and EGF we could able to redirect MSCs into neuro-ectodermal fate. The cellular fate change was assessed by the ability for the formation of neuro-sphere, genes and protein expression. Apart from down- regulation of MSCs-related genes (*ACTA2*, neucleostemin, vimentin and fibronectin), these cells showed several folds increase of neuro-ectodermal specific gene

expressions, such as *SOX1*, *SOX2*, *PAX6*, musashi1, along with neuron specific transcription factors *NEUROD1*, *NEUROD2* and *NEUROG2*. Further, fate change was confirmed by specific protein expression.

C. Stem-ness and cancer progression

Gene ontology analyses of microarray results showed that ascitic fluid-derived $EpCAM^+CD45^+$ cells over-expressed 12 categories of cancer associated genes as compared with $EpCAM^+$ tumor cells. *In vitro* functional analysis confirmed that this hemato-epithelial cell type acquired resistance against paclitaxel and cisplatin drugs separately or in combination. Though putative cancer stem cells ($CD133^+$ and $CD117^+CD44^+$) constituted a minor fraction of $EpCAM^+CD45^+$ cells, the entire population showed highly invasive due to activation of mesenchymal-epithelial transition. $EpCAM^+CD45^+$ tumor cells highly expressed MHC class I antigen thereby was able to evade natural killer cells-mediated immune surveillance, just opposite results were observed in the case of $EpCAM^+$ tumor cells. Further, it was revealed that exosomes secreted by non-tumor cells of the ascitic fluid were able to induce drug resistance and invasiveness in OVCAR5 cell line.

Publications

Original peer-reviewed articles

1. Kumar A, Dudhal S, ASundari T A, Sunkara M, Usman H, Varshney A, Mukhopadhyay A*(2016) Dopaminergic-primed fetal liver mesenchymal stromal-like cells can reverse parkinsonian symptoms in 6-hydroxydopamine-lesioned mice. *Cytotherapy* **18**: 307-319.
2. Baligar P, Mukherjee S, Kochat V, Rastogi A, Mukhopadhyay A* (2016) Molecular and cellular functions distinguish superior therapeutic efficiency of bone marrow CD45 cells over mesenchymal stem cells in liver Cirrhosis. *Stem Cells* **34**:135-147.
3. Kochat V, Kanjirakkuzhiyil S, Baligar P, Nagarajan P, Mukhopadhyay A (2015) Donor antigen-primed regulatory T cells permit liver regeneration and phenotype correction in hemophilia A mouse by allogeneic bone marrow stem cells. *Stem Cell Res Ther.* **6**:129.
4. Sahu N, Baligar P, Midha S, Kundu B, Bhattacharjee M, Mukherjee S, Mukherjee Souhrid, Maushart F, Das S, Loparic M, Kundu SC, Ghosh S, Mukhopadhyay A (2015) Nonmulberry silk fibroin scaffold shows superior osteoconductivity than mulberry silk fibroin in calvarial bone regeneration. *Adv Healthc Mater.* **4**:1709-1721.

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Chandrima Shaha

Cell Death Regulation

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Regulatory networks driving cell fate decisions are important to investigate in the context of understanding diseases. The overall theme of the research program is to elucidate the processes that influence cell death programs under varying physiological conditions in diverse model systems. Broadly, we explore the underlying mechanisms of cell survival and death in diverse intracellular and extracellular conditions using a lower eukaryotic cell, the protozoan parasite *Leishmania* and the higher eukaryotic mammalian carcinoma cells.

A. Cell death in protozoan parasites

As alluded to in last years' report, we continued our efforts to understand the role of host molecules in sustaining *Leishmania* infection. Our studies show an increase in anti-apoptotic members and a decrease of proapoptotic proteins of the Bcl-2 family post infection, suggesting pathogen induced effect on this family of proteins. Manipulation of levels of the anti-apoptotic members contributes to parasite clearance on downregulation and parasite sustenance on overexpression. We suggest the use of small molecule inhibitors for Bcl-2 family of proteins as anti-leishmanial agents. In another study, we show that host microRNA *MIR-30A-3p* increases in response to infection. Its inhibition leads to an autophagic response with increased cellular autophagy and a reduction in parasite burden. We also show in a related investigation that fatty acids with longer carbon chain length and a higher degree of unsaturation are more potent in inducing apoptosis in the *Leishmania* parasites.

B. Mechanisms underlying cell death in cancer

The damaged mitochondria within cells undergo mitophagy for elimination. This is an important aspect for cellular homeostasis. Our studies show the novel observation of stress inducible antioxidan proteins sestrins

regulating the function of vital proteins like PINK1 and parkin required to precipitate mitophagy. This is related to cell survival suggesting an important role of sestrins in cell viability. In another study, reporting further on the behaviour of the mTOR complex in embryonal carcinoma cells, we show that levels of mTOR and its downstream components like Raptor, PRAS40, GβL, Rag C and Rictor increase in the absence of constitutive levels of p53. We show a novel observation that mTORC2 regulates mTORC1 through p53 and both mTORC1 and C2 are important for cell survival.

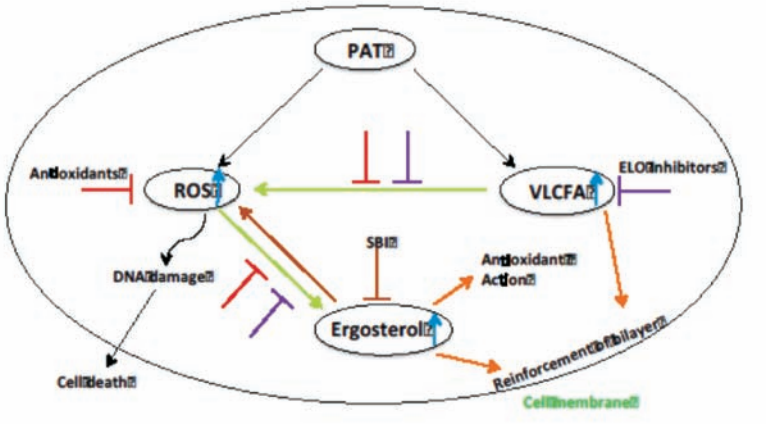

Figure legend: Schematic drawing showing possible interaction of the different cellular components during drug treatment in the *Leishmania* parasite. Drug treatment leads to ROS increase, ergosterol and VLCFAs (blue). VLCFAs increase ROS leading to increased ergosterol (green). Antioxidants inhibit ROS production by drug and VLCFAs thus inhibiting ergosterol increase (red). VLCFA inhibitors decrease ergosterol. Sterol biosynthesis inhibitors downregulate ergosterol levels and induces an increase in oxidative stress (brown). Ergosterol can reinforce cell membrane or at an elevated level act as an antioxidant (orange).

Publications

Original peer-reviewed articles

1. Jagadish N, Parashar D, Gupta N, Agarwal S, Purohit S, Kumar V, Sharma A, Fatima R, Topno A P, Shaha C, Suri A (2015) A-kinase anchor protein 4 (AKAP4) a promising therapeutic target of colorectal cancer. *J Exp Clin Cancer Res* **34:**142**.**
2. Mathur R, Das RP, Ranjan A, Shaha C (2015) Elevated ergosterol protects *Leishmania* parasites against antimony-generated stress. *FASEB J* **29** :4201-4213.
3. Rajaraman P, Dey B, Majumder PP, Mayor S, Pillai MR, Ramaswamy S, Shaha C, Johnson M, Sivaram S, Trimble EL, Harlow EE, VijayRaghavan K (2015) First International Workshopson Provocative Questions (PQ) in Cancer Research, October-November 2014, New Delhi, Bengaluru, and Thiruvananthapuram, India**.** *J Cancer Policy* **6:**33-36.

Satish Kumar Gupta

Cellular and molecular aspects of reproduction and viral infection

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Development of contraceptive vaccine

Immunization of female FvB/J mice with *E. coli*-expressed recombinant protein encompassing promiscuous T-cell epitope of bovine RNase followed by dilysine linker, mouse Sp17 C-terminal fragment (aa residues 76-126), dilysine linker, T cell epitope of tetanus toxoid and two repeats of gonadotropin releasing hormone resulted in failure of 80% of the immunized animals to conceive. Interestingly, hundred percent female mice failed to conceive when immunized female mice were mated with the male mice also immunized with this recombinant protein.

Molecular mechanisms associated with migration, invasion, and differentiation of the trophoblastic cells

i) Trophoblastic cell migration: Using siRNA, the role of Wnt4 and Wnt11 during hepatocyte growth factor (HGF)-mediated HTR-8/SVneo trophoblastic cell migration in scratch wound assay was demonstrated. Treatment of HTR-8/SVneo cells with HGF also led to a significant increase in the expression of Intα2, Intα6 & IntαV.
ii) Trophoblastic cell invasion: Toward the understanding of IFNγ-mediated decreased trophoblast invasion, 18 differentially up-regulated genes observed by deep sequencing were also confirmed by qRT-PCR. The effects of

silencing BATF2 and CD74 by siRNA on IFNγ-mediated invasion are in progress.

Increased invasion of HTR-8/SVneo trophoblastic cells by EGF is associated with phosphorylation of Erk½, STAT3 and STAT1. Inhibition of Erk½ phosphorylation by the inhibitor (U0126) led to a decrease in EGF-mediated invasion and inhibition of STAT3 and STAT1 phosphorylation, suggesting a cross-talk between Erk and JAK-STAT pathways. However, STAT3 silencing by siRNA (Fig. 1A, B), though led to inhibition of EGF-mediated invasion (Fig. 1C), but had no effect on Erk½ phosphorylation as compared to control siRNA treated cells (Fig. 1A, D). As STAT3 silenced cells had no significant decrease in the phosphorylation of Erk½, STAT3 activation is essential for EGF-mediated increase in the trophoblast invasion.

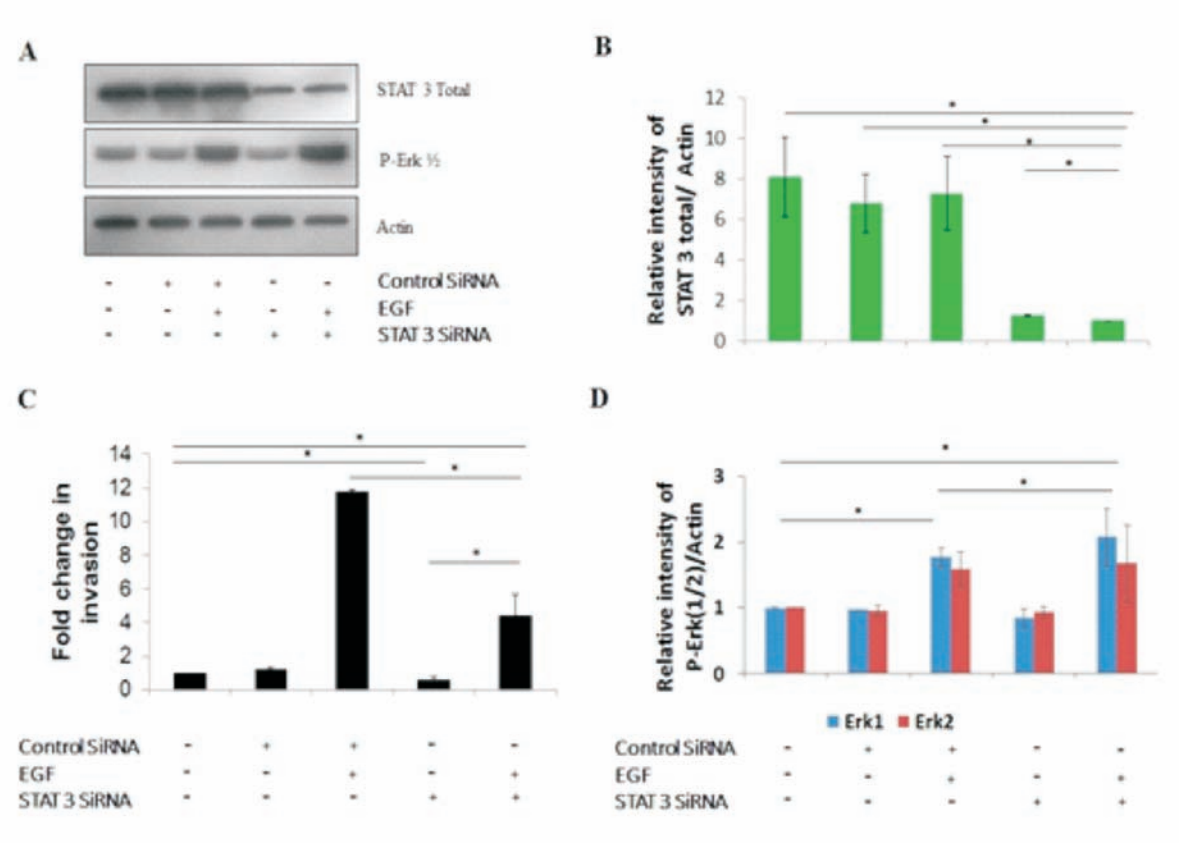


Fig. 1: Effect of STAT 3 silencing on invasion of trophoblastic cells. HTR-8/SVneo cells were silenced for STAT3 by siRNA and silencing assessed by Western blot. Its effect on EGF mediated increase in invasion was studied in matrigel invasion assay. **Panel A** shows the representative blots of STAT3 silencing and **Panel B** shows the densitometric profile of total STAT3 represented as mean ± s.e.m. of three independent experiments. **Panel C** shows the effect of STAT3 silencing on invasion in presence or absence of EGF. **Panel D** shows the densitometric profile of Erk½ phosphorylation with respect to actin in different experimental groups. Values in panels A, B, C, and D represent mean ± s.e.m. of three independent experiments.

In addition, the role of oxidative stress on the invasion of trophoblast cells was investigated. The reactive oxygen species generated by 1, 10 and 25 µM of H_2O_2 had no significant changes in the viability as well as the proliferation of the cells. However, a significant increase in the invasion of the trophoblast cells was observed.

iii) Trophoblastic cell differentiation: SiRNA-mediated Wnt10b silencing led to a decrease in forskolin-/hCG-mediated BeWo cell fusion. Decrease in secreted hCG was observed in forskolin-treated Wnt10b knocked-down cells (Fig. 2). Wnt10b silenced cells also showed a decrease in the nuclear and cytoplasmic β-catenin as well as cellular GCMa & syncytin-1 expression. These observations suggest the significance of Wnt10b in trophoblastic BeWo cells fusion via activation of β-catenin/GCMa/syncytin-1 pathway.

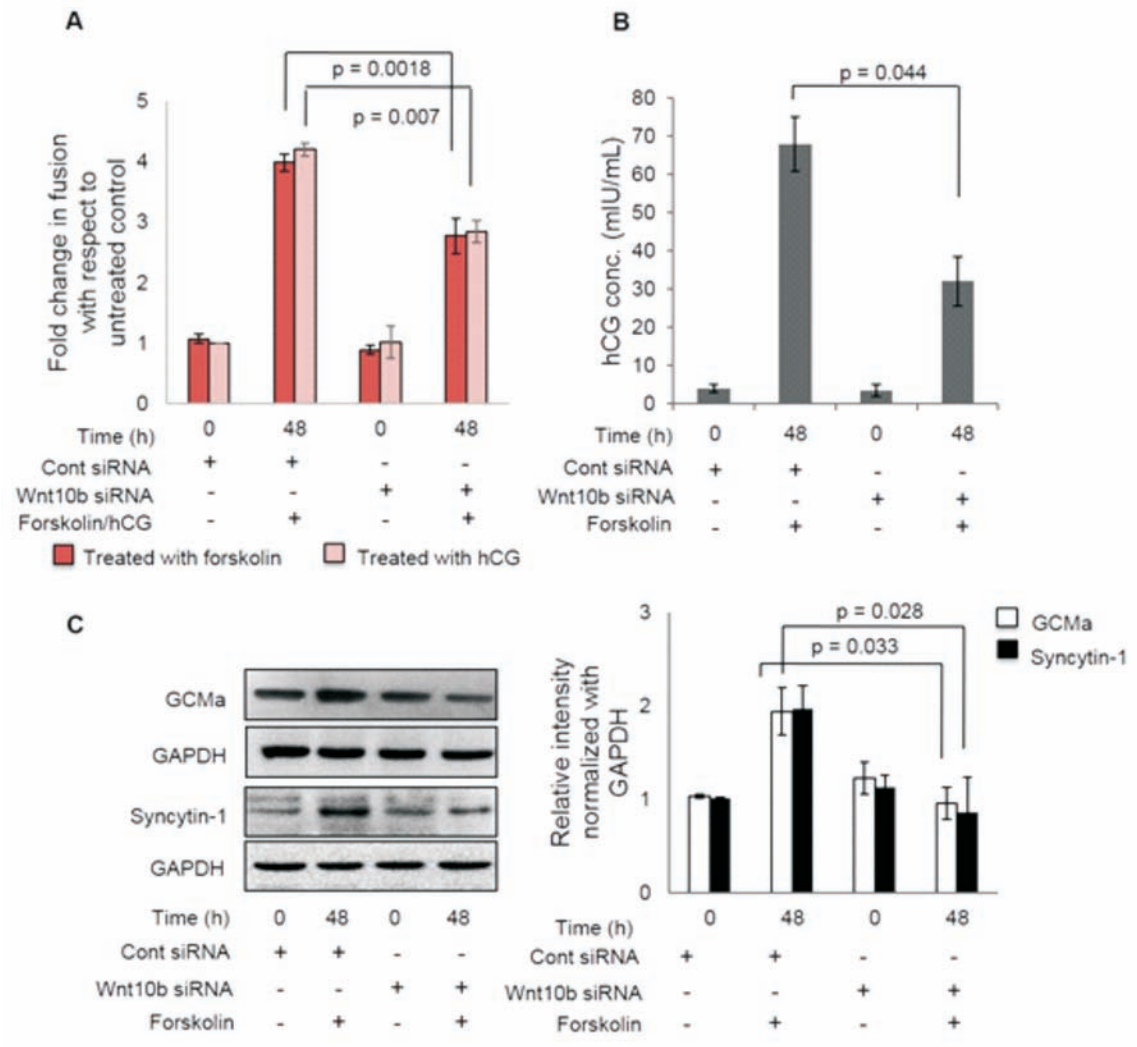


Fig. 2: Effect of Wnt10b silencing on forskolin-/hCG-mediated BeWo cell fusion. BeWo cells were silenced for Wnt10b using siRNA. Forskolin-/hCG-mediated BeWo cell fusion was studied at 0 and 48 h by desmoplakin I+II staining and hCG secretion in forskolin-treated cells was analyzed by ELISA. **Panel A** shows fold change in forskolin-/hCG-mediated cell fusion with respect to control siRNA at 0 h. **Panel B** shows hCG secreted by control and Wnt10b silenced cells in response to forskolin treatment at 0 and 48 h. **Panel C** represents comparative expression profile of GCMa and Syncytin-1 by Western blot in control and Wnt10b silenced cells on treatment with forskolin. GAPDH was used as an internal control. Values in panels A, B, and C represent mean ± s.e.m. of three independent experiments.

Herbal formulation for prevention of HIV-1 and HSV-2 infection

In collaboration with HLL lifecare India Ltd, Thiruvananthapuram, an aqueous gel-based formulation comprising of the 50% ethanolic extracts from four plants was prepared, which showed dose dependent inhibition of HIV-1 infection. In addition, the herbal formulation also exhibited anti-HSV-2 activity at different stages of viral infection in plaque reduction assay. The pre-clinical safety studies revealed that herbal formulation at 1 mg/ml had no significant changes in the viability of 4 different strains of lactobacilli and human cervico-vaginal keratinocyte cells (VK2/E6E7), hemolytic activity, the transepithelial resistance of monolayer formed by Caco-2 epithelial cells, and secretion of proinflammatory cytokines by VK2/E6E7 cells.

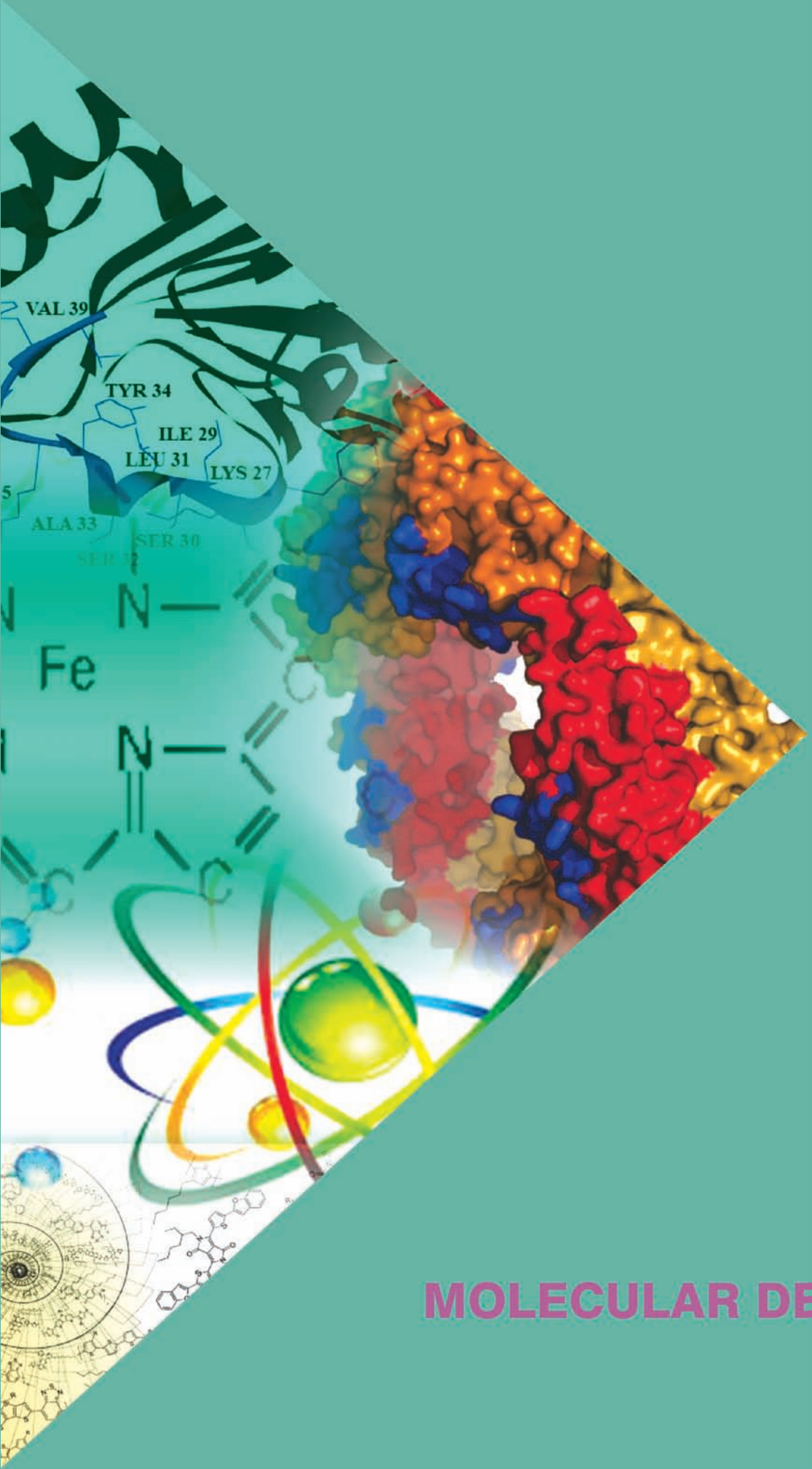
Publications

Original peer reviewed articles

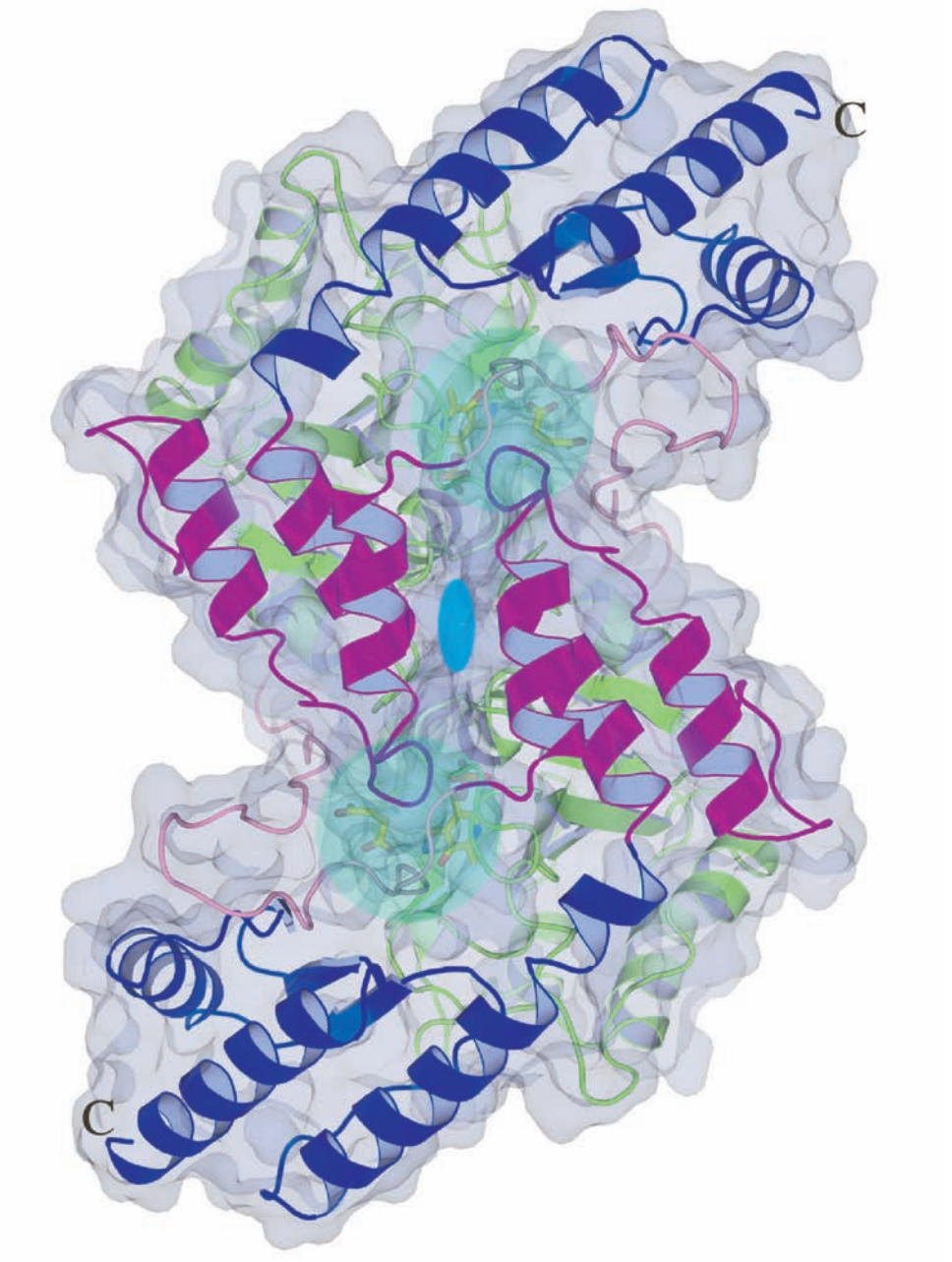
1. Srivastava A, Agarwal A, Gupta SK, Jain N (2016) Graphene oxide decorated with Cu(I)Br nanoparticles: A reusable catalyst for the synthesis of potent bis(indolyl)methane based anti-HIV drugs. *RSC Adv* **6**:23008-23011.
2. Joonè CJ, Bertschinger HJ, Gupta SK, Fosgate GT, Arukha AP, Minhas V, Dieterman E, Schulman ML (2015) Ovarian function and pregnancy outcome in pony mares following immunocontraception with native and recombinant porcine zona pellucida vaccines. *Equine Vet J* doi: 10.1111/evj.12557. (In Press)
3. Arukha AP, Minhas V, Shrestha A, Gupta SK (2016) Contraceptive efficacy of recombinant fusion protein comprising zona pellucida glycoprotein-3 fragment and gonadotropin releasing hormone. *J Reprod Immunol* **114**: 18-26.
4. Laishram S, Moirangthem DS, Borah JC, Pal BC, Suman P, Gupta SK, Kalita MC, Talukdar NC (2015) Chrysin rich *Scutellaria discolor* Colebr. induces cervical cancer cell death via the induction of cell cycle arrest and caspase- dependent apoptosis. *Life Sci* **143**: 105-113.
5. Malik A, Mallajosyula VV, Mishra NN, Varadarajan R, Gupta SK (2015) Generation and characterization of monoclonal antibodies specific to avian influenza H5N1 hemagglutinin protein. *Monoclon Antib Immunodiagn Immunother* **34**: 436-441.
6. Debnath U, Verma S, Singh P, Rawat K, Gupta SK, Tripathi RK, Siddiqui HH, Katti SB, Prabhakar YS (2015) Synthesis, biological evaluation and molecular modeling studies of new 2,3-diheteroaryl thiazolidin-4-ones as NNRTIs. *Chem Biol Drug Des* **86**: 1285-1291.
7. Malhotra SS, Suman P, Gupta SK (2015) Alpha or beta human chorionic gonadotropin knockdown decrease BeWo cell fusion by down-regulating PKA and CREB activation. *Sci Rep* **5**: 11210.

Reviews/Proceedings

1. Gupta SK, Malik A, Arukha AP (2015) Ovarian and oocyte targets for development of female contraceptives. *Expert Opin Ther Targets* **19**: 1433-1446.
2. Gupta SK (2016) Embryo implantation and pregnancy: Immunologic intricacies and strategies for its success. *Am J Reprod Immunol* **75**: 205-207.
3. Gupta SK, Malhotra SS, Malik A, Verma S, Chaudhary P (2016) Cell signaling pathways involved during invasion and syncytialization of trophoblast cells. *Am J Reprod Immunol* 75: 361-371.



MOLECULAR DESIGN



Dimeric 3D structure of Mycobacterium tuberculosis ArAT in cartoon and surface representations.

MOLECULAR DESIGN

Monica Sundd

Structural studies on proteins, dynamics and ligand interactions using NMR

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Usha Yadav
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The theme of our research is to understand the structure, backbone dynamics, and interactions of proteins using NMR and other biophysical techniques. We are focusing on some of the key proteins involved in the fatty acid metabolism of *Leishmania viz*. the acyl carrier protein, 4'-Phosphopantetheinyl transferase *etc.*

Understanding the structure and function of the type II fatty acid biosynthesis pathway of *Leishmania major*

Based on the enzyme kinetics studies of LmACP with AcpS and Sfp, we previously hypothesized that the interaction interface of LmACP might have evolved convergently with its cognate PPT, a group II 4'-phosphopantetheinyl transferase (UniProt ID Q4QCW3).

4'-phosphopantetheinyl transferase of *L. major* (LmPPT) does not show any catalytic activity towards its cognate ACP.

Surprisingly, only 18% conversion of the wild type LmACP and the mutants N35D, F44M, N35D+F44M, to the holo-form was observed after 8 hrs incubation (Figure 1A, lanes 1-8). Interestingly, an assay carried out in the absence of LmPPT, lane 9) also displayed equivalent conversion to holo-LmACP.

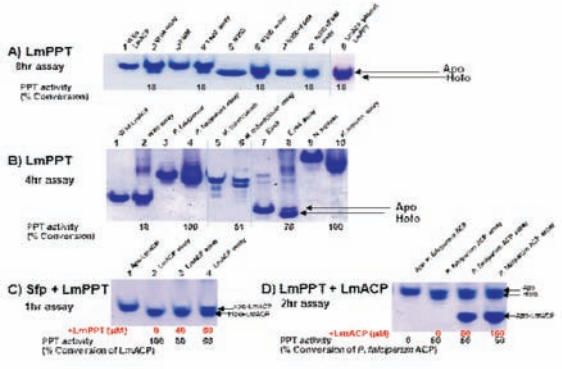


Figure 1. *In vitro* conversion of A) LmACP and its mutants using LmPPT, B) heterologous ACPs (type I, and type II) using LmPPT, C) LmACP by Sfp in the presence of LmPPT D) *P. falciparum* ACP by LmPPT in the presence of LmACP.

LmPPT efficiently converts heterologous type I and type II ACPs into their holo-form.

The rate of phosphopantetheinylation of other type I and type II ACPs by LmPPT was also followed. An equivalent amount of the enzyme, under the same assay conditions, displayed higher activity towards *H. sapiens* ACP (71% conversion), *P. falciparum* ACP (70%), *M. tuberculosis* (35%), and *E. coli* ACP (45% conversion) after 3hrs incubation, as shown in Figure 1B.

LmACP forms a relatively tight complex with LmPPT.

The chemical shift changes upon binding of LmACP with its cognate PPT (unlabeled) were similar to those observed with Sfp. Interestingly, a few more amides in the helix II, loop II and helix III of LmACP *viz.* Phe 44, Ile 46, Asp 53, Asp 58, Ile 62 and Gln 63 displayed a noticeable chemical shift change, in addition to Glu 15 and Ile 54 HN suggesting a tight complex, as shown in Figure 2.

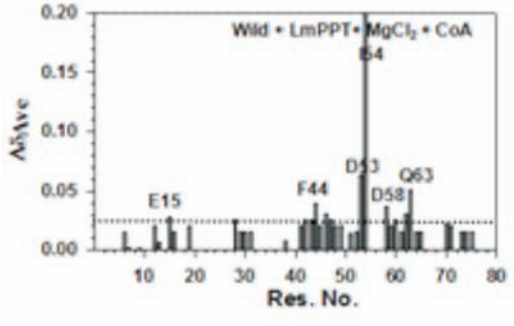


Figure 2. Average amide chemical shift changes of LmACP upon binding to LmPPT in the presence of 2mM Mg^{2+} and 1.5mM CoA.

Sfp catalyzed the conversion of apo-LmACP is significantly reduced in the presence of LmPPT.

Approximately, 20% reduction in a holo-LmACP product was observed in the presence of one fold molar excess (Figure 1C, lane 3) and 40% reduction in two fold excess of LmPPT (Figure 1D, lane 4).

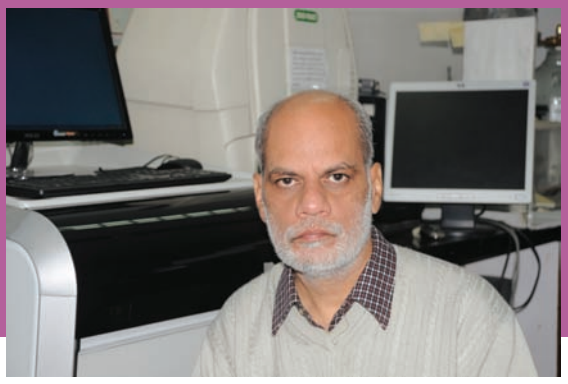
LmACP does not affect the conversion of non-cognate ACPs by LmPPT.

LmPPT assays were carried out using *E. coli* and *P. falciparum* ACP as substrates (40M), in the presence of LmACP. No change in the *P. falciparum* holo-ACP product was observed in the presence of 2 fold or 4 fold molar excess of LmACP (Figure 1D, lanes 3 & 4).

Publication

Original peer reviewed article

1. Kumar A, Arya R, Makwana PK, Dangi RS, Yadav U, Surolia A, Kundu S, Sundd M (2015) The structure of the holo-acyl carrier protein of *Leishmania major* displays a remarkably different phosphopantetheinyl transferase binding interface. *Biochemistry* **54**:5632-5645.



Pramod K. Upadhyay

To develop strategies for making sensors and actuators for biological processes

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Research Fellow
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Hepatocytes like cells from PBMCs

Global transcriptome analysis for monocytes, RM, NeoHep and human hepatocytes

We were able to de-differentiate peripheral blood mononuclear cells (PBMCs) to Reprogrammed monocytes (RM) which were differentiated to hepatocyte like cells (NeoHep). The differentially expressed genes and associated processes in monocytes, RM and NeoHep were examined. The FPKM values of the genes for each sample was converted to logarithmic base 10 value. Using these values a hierarchical clustering of these samples based on their gene expression was performed using Spearman's rank correlation matrix and the dendrogram obtained is shown in Figure 1A.

In this dendrogram, the H_Monocytes (H : Healthy) and HNP_Monocytes (HNP : HBsAg-NAT Positive) were in the same cluster. Similarly H_NeoHep and HNP_NeoHep were also in the same cluster. However, the H_RM and HNP_RM were in different clusters. The node height for NeoHep cluster (0.068) was closer to the node height of Hepatocyte (0.187) in comparison to that of H_RM (0.033) and Monocyte cluster (0.0). The node height of HNP_RM (0.584) was distant from the remaining six samples.

The heat map (Figure 1B) displays the differential expression of hypoxia related genes during the reprogramming of healthy and HNP monocytes. There were many common up-regulated hypoxia related genes in H_RM and HNP_RM. Similarly, there were a large number of common up-regulated metabolism related genes (Figure 1C) in H_NeoHep and HNP_NeoHep but the up-regulated genes in RM were not common. A noteworthy down-regulation in inflammation and immune defence related gene expression was observed in RM and NeoHep as shown in the heat maps (Figure 1D). A summarized overview of differential expression of genes is given in Figure 1E.

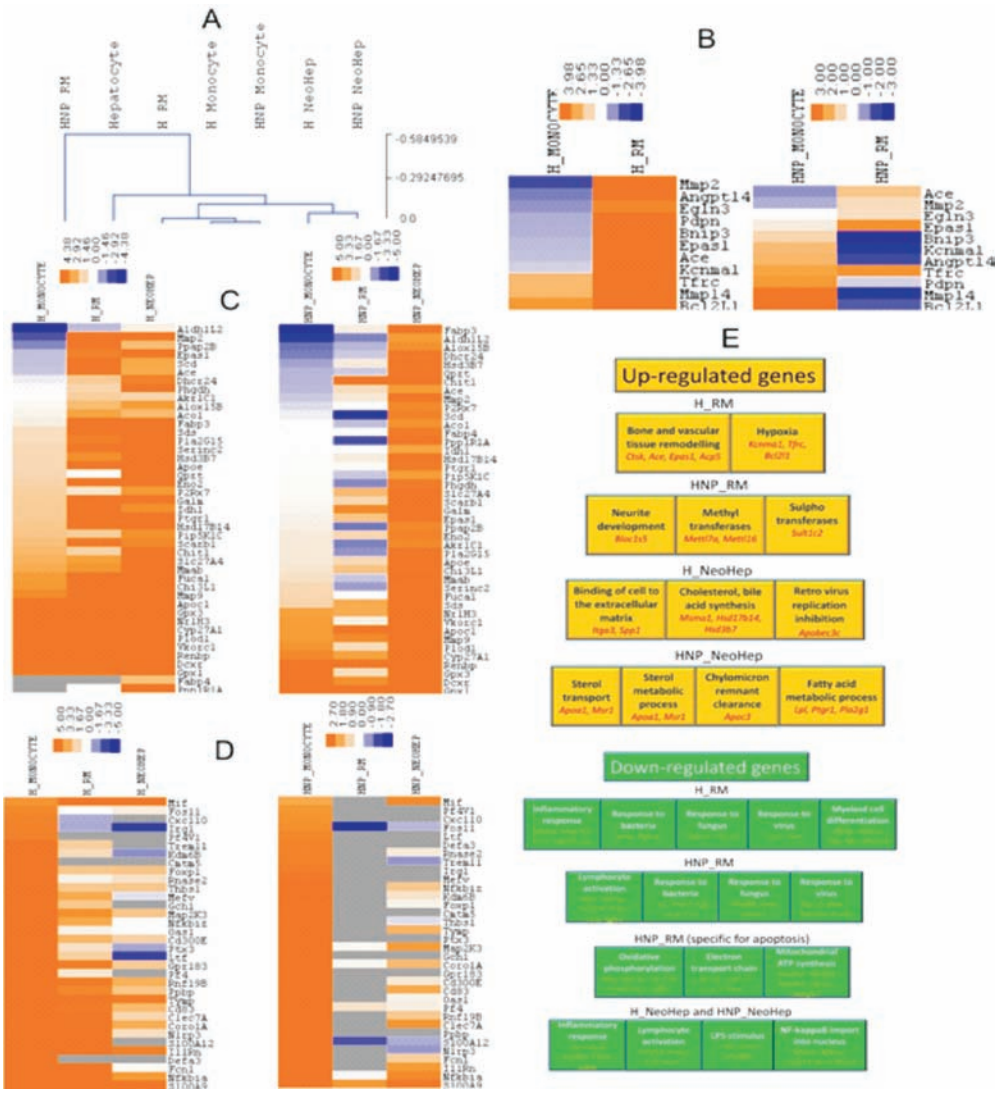


Figure 1 : Differential expression of genes during reprogramming and differentiation of monocytes. Panel (A) dendrogram of hierarchical clustering using Spearman's Rank Correlation matrix. Heat maps of genes involved in hypoxia expressed in monocyte and RM are shown in Panel (B) cholesterol and bile acid metabolism expressed in monocyte, RM and NeoHep in Panel (C) The expressions of down regulated genes related to inflammation and immune response in RM and NeoHep in comparison to monocytes are shown in Panel (D) Panel (E) summary of differential expression of genes and corresponding biological process.

Differentiation of PBMCs into retinal neuron like cells (RNLCs)

We are developing a novel method for *in vitro* retinal neurogenesis employing the use of easily and non-invasively available peripheral blood derived monocytes.

The differentiated RNLCs express markers corresponding to a diverse group of retinal cells (Figure 2), which were absent in monocytes and RM, indicating that RNLCs as a composite culture of several types retinal cells.

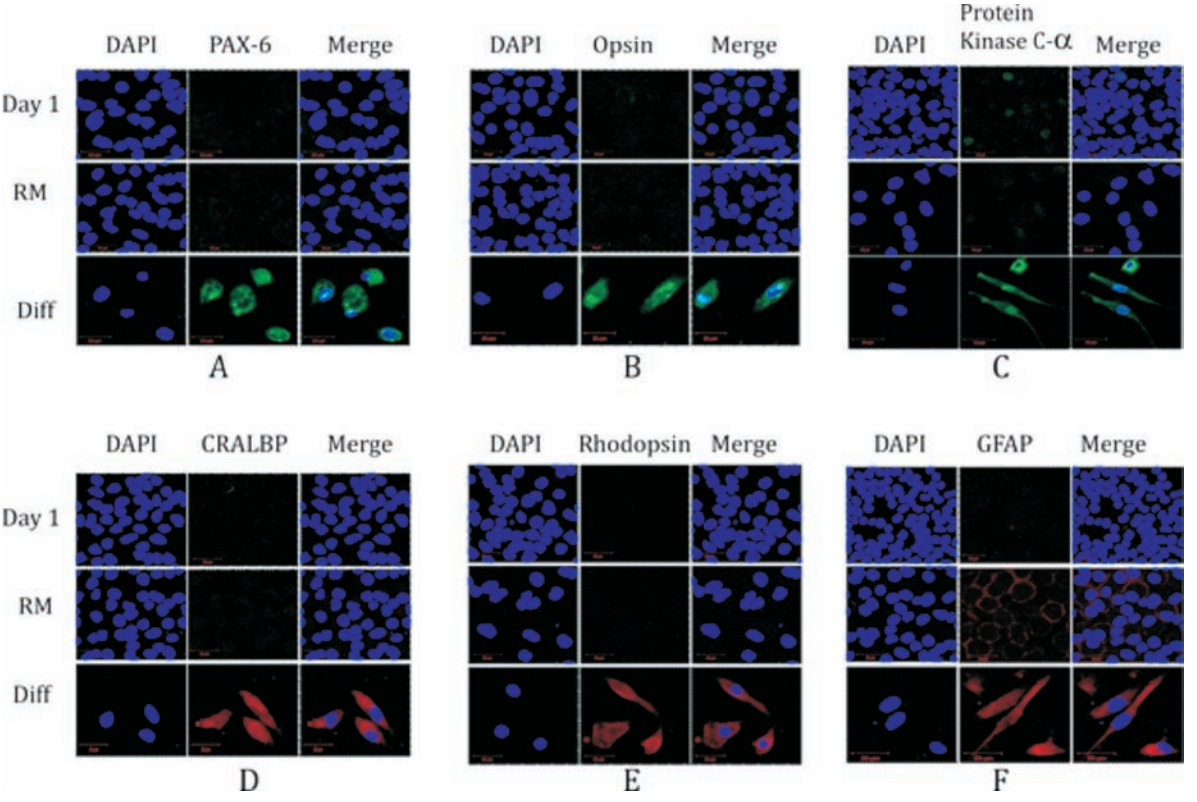
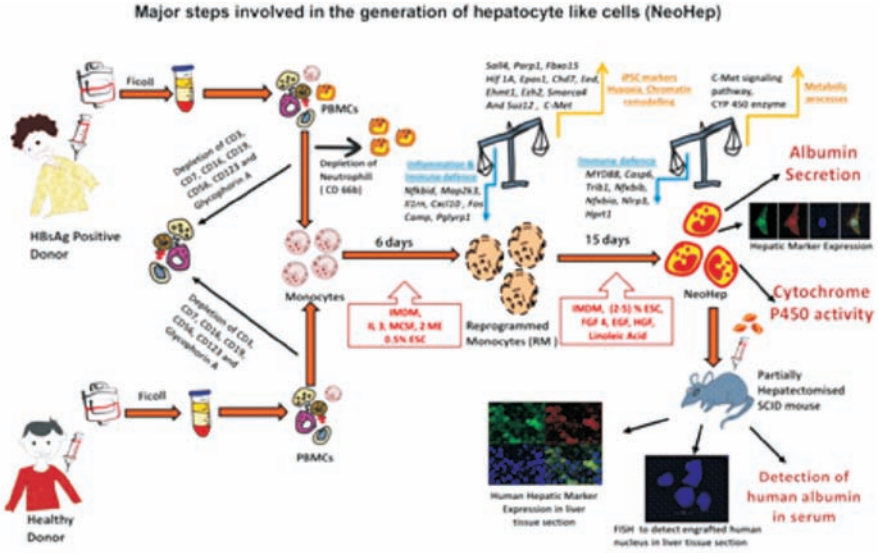


Figure 2 : Confocal images showing the expression of retinal markers (A) PAX-6 : common retinal marker (B) Opsin : cone photoreceptor marker (C) Protein Kinase C-alpha : bipolar cells (D) Cellular Retinaldehyde Binding Protein (CRALBP) : retinal pigment epithelial cells and muller cells (E) Rhodopsin : rod photoreceptors (F) Glial Fibrillary Acidic Protein(GFAP) : glial cells. Green : Alexa fluor 488 stained; Red: Alexa fluor 594; Blue : DAPI

Publications

Original peer-reviewed articles

1. Jain Y, Upadhyay P (2015) Tuberculosis burden at post mortem. *Lancet Infect Dis.* **15**:1132-1133.
2. Iyer S, Upadhyay P, Majumdar SS, Nagarajan P (2015) Animal models correlating immune cells for the development of NAFLD/NASH. *J Clin Exp Hepatol* **5**:239-245.



Preparing grounds for autologous cell based therapy for liver disease due to hepatitis B infection.
A cartoon showcasing the major steps involved in the generation of hepatocyte like cells (NeoHep) from healthy and HBsAg-NAT positive (HNP) blood.

Rajendra P Roy

Protease-catalyzed splicing of peptide bond

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We study the principles underlying enzymatic peptide ligation reactions with a view to applying them to the semi-synthesis of proteins and assembly of defined bioconjugates. Transpeptidase sortase that catalyzes covalent anchoring of surface proteins to the cell wall in gram-positive bacteria has turned out to be an extremely useful enzyme in this endeavour. The propensity of sortases to ligate native or engineered polypeptide scaffolds offer unprecedented opportunities in synthetic protein chemistry.

Structure, dynamics and function of Sortases

Newer challenging applications of sortases would require the availability of enzymes with newer specificities. Detailed biochemical and structural elucidation of different types of sortases may be necessary for the rational design of substrate tolerance. Towards this, we have been delineating structure-function interrelationships in a variety of sortases.

The *Streptococcus pneumoniae* SoraseA (Sp-SrtA) forms intertwined domain swapped dimers in crystals. Sp-SrtA also predominantly exists as a dimer in solution. However, the question remains whether the "swapped" dimer is a catalytically active structure of the enzyme. We have begun to address the fidelity/relevance of the domain swapped structure by generating hybrid dimers composed of wild type enzyme and an inactive mutant. For this, wild type and the mutant enzyme were cloned with disparate affinity tags [His tag for active enzyme and Strep-tag for the mutant] in the same pET duet vector. The clone was transformed into Bl21 *E. coli* and expressed. The isolation process of the heterodimeric protein using sequential purification by Ni-affinity and Strep-Tactin affinity chromatography is in progress. In another approach, attempts are being made to isolate the hybrid dimer from a mixture of individual proteins expressed separately with orthogonal affinity tags.

Bioinformatic analysis of the recently published genome of *S. avermitilis* predicts the presence of at least four ClassE Sortases (SrtE) and 13 putative substrates, all of which contain a LAXTG motif near the C- terminus. We had previously obtained a truncated version of one the predicted class E sortase and a new sortase from *Thermobifida fusca* genome, classified as SrtF. The substrate specificities of these enzymes were extensively characterized.

Sortase-mediated protein labeling and conjugation

We reported conceptualization of a sortase-mediated strategy for the semisynthesis of SUMO protein conjugates that can serve as a substrate for desumoylating enzymes. Toward this, SUMO clones were suitably modified for expression of a protein compatible with sortase specificity. Appropriate peptides of different chain length from p53 containing target site for sumoylation were generated using Fmoc protocol exploiting an orthogonal protection at Lysines.

We first explored the feasibility of SUMO semisynthesis strategy with a short C-terminus SUMO peptide sequence and a model peptide derived from tp53 (arget sequence). The transpeptidation reaction of the model peptide with the SUMO sequence proceeded smoothly yielding the desired 17-mer product. Subsequently, engineered SUMO proteins were expressed with a C-terminus His-tag. The individual purified proteins when reacted with the p53 target peptide in the presence of sortase produced a conjugate in much the same way as the aforementioned SUMO peptide establishing Sortase-mediated ligation as a useful tool for the semi-synthesis of SUMO conjugates.

Sarika Gupta

Therapeutic Interventions in Chronic Diseases

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Interests of the group lie in identifying underlying principles in a disease pathogenesis to discover new targets, designing molecular intervention strategies and confirming the biological/therapeutic activities of the designed compounds. The small molecule regulators contribute to both drug development and understanding biological systems in human body.

1. To study the role of BMPs in the regulation of glucose homeostasis, development of obesity and insulin resistance.

Diabetes mellitus is a chronic metabolic disease, characterized by the inability to maintain blood glucose concentrations within physiological limits. In Type II Diabetes (DM-II), insulin target tissues, such as skeletal muscle, liver, and adipose, progressively become resistant to effects of circulating insulin. A direct link between the development of insulin resistance and presence of chronic inflammation, in a case of obesity exists, with cytokines playing an important role in glucose metabolism. Both insulin and BMP signalling pathways are essential for adipose tissue development, and a cross-talk between these two crucial pathways in adipose and other insulin target tissues might result the in coordination of energy balance. In an attempt to elucidate this mechanism of crosstalk by BMPs in modulating insulin signalling and glucose uptake, studies were performed in primary insulin responsive tissues. We observed an age dependent increase in serum BMP-4 and decrease in serum BMP-7 levels in animal models of Type II diabetes. In this study, our data demonstrates a novel and direct role of BMP - 4 and 7 in the regulation of glucose homeostasis and insulin resistance. BMP- 7 in diabetic mice was able to restore glucose

uptake in cells with attenuated insulin signalling, thereby improving metabolic parameters. BMP-4 on the contrary inhibited insulin signalling in insulin target tissues resulting in insulin resistance.

2. Triphenylmethane dyes as an inhibitor of wild type and mutant alpha-synuclein aggregation and modulator of neurotoxicity

Inhibition of amyloid formation along with modulation of toxicity employing small molecules is emerging as a potential therapeutic approach for protein misfolding disorders which include Parkinson's disease, Alzheimer's disease and Multiple System Atrophy etc.. As an extension of the project presented last year, we tested the inhibitory effect of two routinely used protein staining dyes viz Coomassie Brilliant blue G (CBBG) and Coomassie Brilliant blue R (CBBR) employing several biophysical and cell based methods. Our results showed that both the dyes not only efficiently inhibit fibrillization but also significantly disrupt existing fibrils. Nonetheless, only CBBR prevented the appearance of A11 epitopes which are the marker of toxicity. Moreover, CBBR was also able to stall fibrillization of A53T mutant α-synuclein and reduce associated neurotoxicity.

Publications

Original peer-reviewed articles

1. Ahsan N, Mishra S, Jain MK, Surolia A*, Gupta S* (2015) Curcumin Pyrazole and its derivative (N-(3-Nitrophenylpyrazole) Curcumin inhibit aggregation, disrupt fibrils and modulate toxicity of Wild type and Mutant α-Synuclein. *Sci Rep.* **5**:9862.
2. Rawat P, Manglani K, Gupta S, Kalam A, Vohora D, Ahmad FJ, Talegaonkar S (2015) Design and development of bioceramic based functionalized PLGA Nanoparticles of risedronate for bone targeting: *In-vitro* characterization and pharmacodynamic evaluation. *Pharm Res.* **32**:3149-3158.

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Molecular mechanism of enzymatic reactions and enzyme-ligand interactions

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Tetramer formation in hGBP1 is associated with the conformational change

To understand whether tetramer formation in wt-hGBP1 is associated with structural change, CD measurements were performed in the absence and presence of either GppNHp or GDP.AlF_4. The CD value at 208 and 220 nm increases with GppNHp compared with the free protein, indicating structural stabilization upon formation of the dimer. Interestingly, with GDP.AlF_4 the CD value decreased more than those with GppNHp, indicating that some of the ordered structures became disordered after the cleavage of the first phosphate, which may be required for the formation of a tetramer. To verify this, similar measurements were done with D103L.D108L (defective in GMP formation). Interestingly, the CD value for the D103L.D108L mutant did not show any change either with GppNHp or GDP.AlF_4. These data suggest that tetramer formation is associated with a structural change of the protein.

To further examine this, intrinsic tryptophan fluorescence measurements of wt-hGBP1 were performed in the absence and presence of the analogues. The fluorescence did not alter with GppNHp, indicating that the microenvironment of tryptophans has not changed upon dimerization. Interestingly, with GDP.AlF_4 the protein displayed a red shift of approximately 6 nm, indicating that tryptophans in the tetramer moved into a relatively polar environment compared with the monomer or dimer. To validate this further, dynamic fluorescence quenching by a known quencher CsCl was performed. K_{sv}(Stern-Volmer quenching constant) was calculated. The values of τ_m derived from the time-resolved fluorescence decay kinetics are 3.6 and 2.3 ns for the absence and presence of GDP.AlF_4, respectively. The value of k_q increased with GDP.AlF_4-bound protein compared with the wild type (2.6±0.2 $x10^9$ vs 1.1±0.1 $x10^9$ $M^{-1}s^{-1}$), confirming that tryptophans in the tetramer are more solvent

exposed compared with the monomer. Collectively, these data indicate that tetramer formation is associated with a conformational change of the protein.

Exchange of the helical domain in hGBP1 with that of hGBP2 leads to the loss of enhanced GMP formation

To understand whether the enhanced GMP formation in wt-hGBP1 is due to tetramerization mediated by the interdomain interactions, two chimeras CH1 and CH2 of hGBP1 and hGBP2 were prepared. In these chimeras the globular domain of wt-hGBP1 was kept intact, but the other two regions were systematically swapped with that of wt-hGBP2, since the catalysis happens only in the globular domain. The overall activity of these chimeras is reduced compared with wt-hGBP1. Interestingly, the ratio of GMP to GDP formation for CH1 was markedly reduced compared with wt-hGBP1 (~ 0.8:1 vs ~ 5.8:1). Like CH1, CH2 also showed a significantly reduced GMP to GDP ratio (~0.85:1). These data show that the substitution of either the helical domain alone or the combination of the helical domain and the intermediate region of wt-hGBP1 with that of wt-hGBP2 significantly decreased the GMP formation compared with the wt-hGBP1, indicating that these chimeras are defective of the enhanced GMP formation in the absence of the helical domain of hGBP1.

Trp159-Asp126 hydrogen bonding interaction in H. pylori arginase is not crucial for positioning the non-conserved motif near the active-site

To evaluate whether Trp159-Asp126 hydrogen bonding interaction is crucial for positioning the motif near the active-site, Asp126Ala mutant was prepared. The fluorescence emission of wild type apo protein displays a peak at 330nm, indicating that the microenvironment of Trp159 is less polar. But, with Co^{2+} ions the emission is blue shifted by 3nm. The apo and holo forms of Asp126Ala mutant showed fluorescence emission similar to that observed for wild type, indicating that the mutation of Asp126 did not change the microenvironment of the tryptophan compared with wild type. These results suggest that the Trp159-Asp126 interaction is not crucial to retain the motif in a hydrophobic environment and thus possibly not important for its positioning.

Janendra K. Batra

Ribonucleases and heat shock proteins: Involvement in host defense

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Theme of research

1. Investigation of the role of human ribonucleases, particularly eosinophil ribonucleases, eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) in host defense. Human ribonucleases are also being explored to design knowledge-based recombinant toxins.

2. Investigation of crucial housekeeping proteins of *M. tuberculosis* for their role in survival and virulence of the pathogen. In this context, functioning of Clp machinery, and RNase P mediated tRNA maturation is being investigated in *M. tuberculosis.*

Molecular mechanism of biological actions of human ribonucleases

In mouse, 15 eosinophil associated ribonucleases (mEARs) have been reported, which have very close similarity to ECP and EDN. We characterized four recombinant mEARs namely, mEAR2, mEAR5, mEAR7 and mEAR11 to understand their mechanism of function. The four mEARs had differential RNase and cytotoxic activities. All four mEARs showed antibacterial and antiparasitic activities. There was no direct correlation in the cytotoxic and antimicrobial activities of the mEARs. It appears that mEARs differ in their membrane interaction and destabilization activities which result in their differential cytotoxic and antimicrobial activities.

Construction of recombinant toxins as potential therapeutics

We have earlier constructed a cytotoxic dimer of human pancreatic ribonuclease (HPR) by linking two monomers by a disulfide bond through two cysteines incorporated in HPR by site directed mutagenesis. To further enhance the cytotoxic activity, we constructed a dimer with a ribonuclease inhibitor (RI) resistant

variant of HPR. The RI-resistant HPR dimer was found to be remarkably more cytotoxic than the wild type HPR dimer on a variety of cell lines. The generated molecule, because of its human origin and high toxicity, should prove to be a useful component of recombinant immunotoxins.

Stress regulation and persistence mechanisms in *Mycobacteria*

We investigated the involvement of ClpB protein in the survival of *M. tuberculosis*. Using ClpB knockout and ClpB knockout complemented with ClpB strains of *M. tuberculosis* we showed that ClpB is required for proper growth of the pathogen under normal condition, whereas it is absolutely essential for survival under heat and hypoxia stress. Our preliminary study with mutants demonstrates that ClpB plays a very crucial role in stress tolerance in *M. tuberculosis*.

We are investigating the mechanism of stress regulation in mycobacteria, particularly the role of a transcriptional repressor, HrcA in stress management. Our studies demonstrate that *M. tuberculosis* HrcA protein requires other protein(s) from the cell to be able to bind to its cognate CIRCE DNA. In a preliminary study, using a hrcA knock out the strain of *M. tuberculosis,* we observed that HrcA is not required for growth and survival of the pathogen under normal conditions.

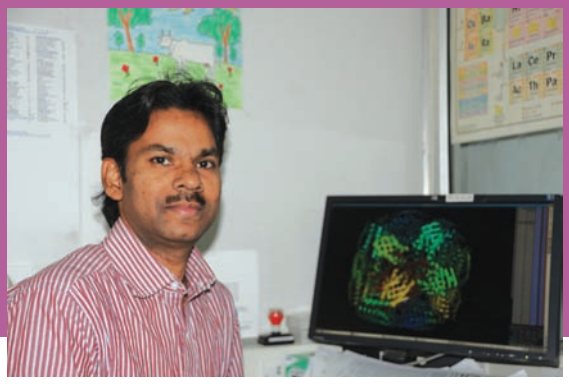
Structure-function analysis of ribonuclease P of *M. tuberculosis*

M. tuberculosis RNase P contains one RNA and one protein subunit. Mycobacterial RNA is larger than typical bacterial RNase P RNAs. It contains the essential core structure with subtle changes along with many unique peripheral elements. Our study demonstrates that in the RNA component of RNase P of *M. tuberculosis,* the P12 helix is important and optimal for RNase P holoenzyme function; helices P15.1 and P18 interact with monovalent and divalent ions, respectively; and P19 helix enhances the structural stability. A homology model of *M. tuberculosis* RNase P RNA indicates many previously unknown new inter- and intra-helical interactions.

Publications

Original peer-reviewed articles

1. Singh A, Ramteke AK, Afroz T, Batra JK (2016) Insight into the role of histidine in RNR motif of protein component of RNase P of *M. tuberculosis* in catalysis. *IUBMB Life* **68**: 178-189.
2. Choudhary S, Pardo A, Rosinke R, Batra JK, Barth, S, Verma RS (2016) Targeting c-kit receptor in neuroblastomas and colorectal cancers using Stem Cell Factor (SCF)-based recombinant bacterial toxins. *Appl Microbiol Biotechnol* **100**: 263-277.
3. Parijat P, Batra JK (2015) Role of DnaK in HspR-HAIR interaction of *Mycobacterium tuberculosis*. *IUBMB Life* **67**: 816-827.
4. Yadav SK, Batra JK (2015) Mechanism of anti-HIV activity of ribosome inactivating protein, saporin. *Protein Pept Lett* **22**: 497-503.



Bichitra K. Biswal

Understanding structures and functions of histidine biosynthesis enzymes from *Mycobacterium tuberculosis*

Ph. D Students

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Khundrakpam Herojit Singh
Abhisek Dwivedi
Bhavya Jha
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Two research projects to decipher structural and functional aspects of proteins from *Mycobacterium tuberculosis (Mtb)*, the organism that causes tuberculosis (TB) in humans. The first one aims at determining and analysing the 3D structures and dissecting the biochemical properties of enzymes of histidine (His) biosynthesis pathway to derive the molecular mechanisms underlying their actions and to design enzyme specific anti-TB small molecule compounds through a structure-guided approach. In the second project, we focus on understanding how *Mtb* membrane associated proteases modulate host factors.

Mtb makes its own histidine (His) from 5-phosphoribosyl-1-pyrophosphate in 10 enzymatic steps by 10 enzymes. His pathway is conserved among bacteria, lower eukaryotes and plants, but is absent from higher eukaryotes including mammals. We have been studying structural, biochemical and inhibition aspects of these enzymes from *Mtb,* largely in the context of deciphering the molecular mechanisms underlying their actions and designing enzyme-specific anti-TB inhibitors. Previously we have determined 3D structures of three enzymes, HisB (imidazole glycerol phosphate dehydratase), HisC (a histidinol phosphate aminotransferase) and HisC2 (an aromatic amino acid aminotransferase) and have characterized these enzymes biochemically. We also have shown structurally and biochemically that a triazole scaffold compounds are competitive inhibitors of HisB. The biological functional unit of HisB is a 24-mer (Figure 1), possessing 432 molecular symmetry.

The overall tertiary structure of HisB, a four-helix-bundle sandwiched between two four stranded β-sheets, resembling the three-dimensional structures of HisB from other organisms. The structure of the HisB-substrate (Imidazole glycerol phosphate; IGP) complex

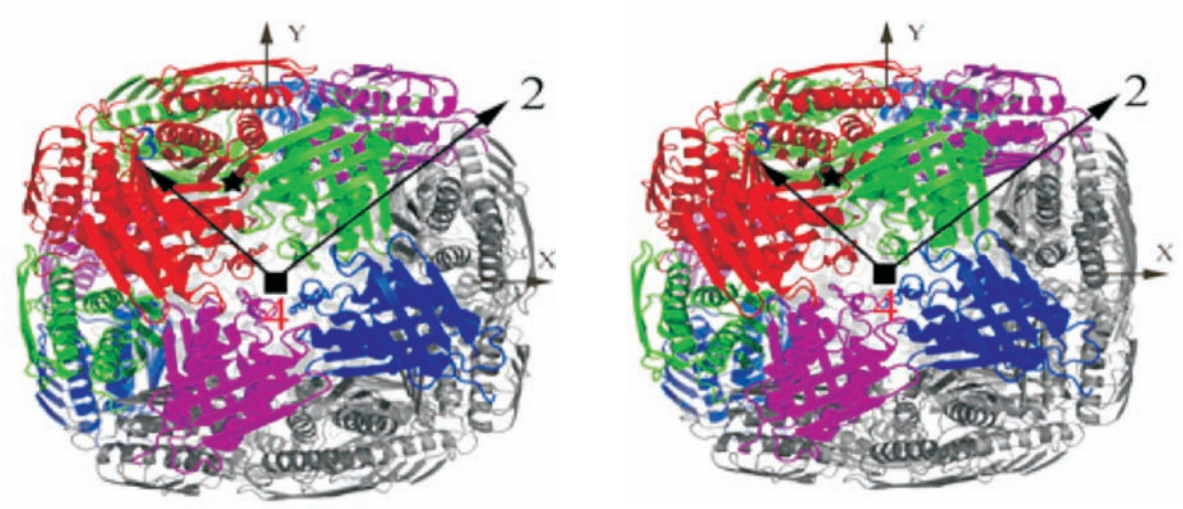

Fig. 1. Stereoview of ribbon representation of 3D structure of HisB. Also shown in the figure is the axes of 432 molecular symmetry.

revealed that the imidazole ring of the IGP is firmly anchored between the two Mn atoms. The rest of the substrate interacts through hydrogen bonds mainly with residues Glu21, Arg99, Glu180, Arg121 and Lys184 which protrude from three separate protomers. And the 24-mer assembly contains 24 identical catalytic centres. Recently, employing structure-based inhibitor design approach we have identified a new triazole derivative compound (triazole-alanine) with inhibitory activity in low micromolar value. The 1.8 Å crystal structure of HisB/triazole-alanine complex demonstrated that traizole-alanine inhibits the function of HisB in a competitive manner. The triazole ring of the inhibitor is placed between the two active site manganese atoms (Figure 2). The complex is stabilized through hydrogen bonding, water-mediated and van der waals interactions. These interactions aid in designing more potent inhibitors. The *in vivo* efficacy of this compound is being evaluated. With regard, the work on membrane proteins, X-ray data from crystals of a truncated version of a membrane protein (Rv2224) were collected and the structure is being solved. In addition, another membrane protein Rv1223 has been prepared and crystallization trials are being carried out.

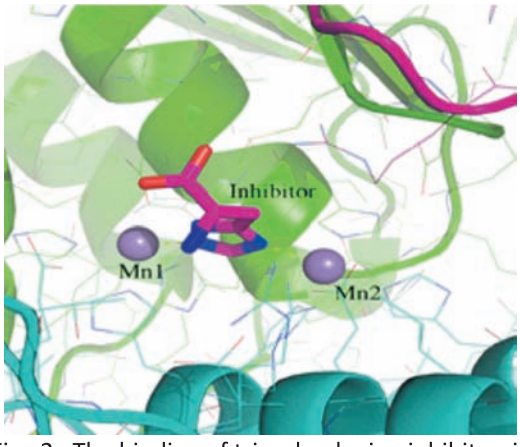

Fig. 2. The binding of triazole-alanine inhibitor in the active site pocket of HisB.

Publication

Original peer-reviewed article

1. Nasir N, Anant A, Vyas R, Biswal BK* (2016) Crystal structures of *Mycobacterium tuberculosis* HspAT and ArAT reveal structural basis of their distinct substrate specificities. *Sci Rep* **6**:18880.

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Debasisa Mohanty

Molecular modelling of proteins and protein-ligand complexes using knowledge-based approaches and all atom simulations

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The objective of the various projects are to investigate, whether the combination of knowledge-based and *ab initio* approaches can be used for (1) predicting substrate specificity of enzymes involved in novel post-translational modifications (2) deciphering substrate specificity of various peptide recognition modules (PRMs) (3) analysis of microRNA-protein interaction networks.

A. Analysis of enzymes associated with novel PTMs

Unlike well known PTMs like phosphorylation, glycosylation, SUMOylation, no bioinformatics resources are available for enzymes associated with novel and unusual PTMs. Therefore, we have developed the novPTMenzy database which catalogs information on the sequence, structure, active site and genomic neighborhood of experimentally characterized enzymes involved in five novel PTMs, namely AMPylation, Eliminylation, Sulfation, Hydroxylation and Deamidation.

AMPylation is a novel post-translational modification (PTM) involving covalent attachment of an AMP moiety to threonine/tyrosine side chains of a protein. Involvement of these novel enzymes in a myriad of biological processes makes them interesting candidates for genome-wide search. Our phylogenetic analysis revealed that this family might have evolved via horizontal gene transfer (HGT). The extensive HGT observed in Fic domains is because it is encoded by highly mobile genomic islands.

B. Analysis of substrate specificity of PTB domains

We have analyzed the sequence and structural features of PTB domains to develop a novel structure based multi-scale approach for deciphering substrate specificity of PTB domains. Explicit solvent molecular dynamics simulations have been carried out on six PTB-peptide complexes for which binding affinities were known from experimental studies. Interestingly, the bound peptide substrate also essentially retained its conformation and orientation in the PTB binding pocket. The binding free energies for various peptides calculated using MM/PBSA showed good correlation with experimental binding energy with correlation coefficient of 0.64. Our simulations could also explain how mutation in the regions flanking the NPxY motif alters contacts with the PTB domain and the binding affinity. This study also demonstrated that based on the binding pockets obtained from MD simulations, the binding energy scores computed using residue based statistical pair potentials shows good correlation with experimental binding energy.

C. Structural analysis of LIN28:pre-let7 complex

LIN28 inhibits biogenesis of miRNA let7 and their expression levels are inversely related. The availability of crystal structure of mouse LIN28A in complex with the precursor element of let-7 has opened up possibilities to study the structural basis of this process. We have performed 200 ns explicit solvent molecular dynamics simulations to identify key specificity determining residues (SDR) important for the inhibitory role of LIN28. We have also used our simulation results to understand why let7-a-3 does not bind to LIN28 as shown in recent experimental studies. Analysis of conservation profile of these SDRs in sequence and structural homologs of LIN28 has revealed that they are evolutionary conserved, thus highlighting their importance in Lin28 activity. We have also analyzed sequence and structures of some pre-miRNAs known to be regulated by LIN28 to emphasize upon additional features in these pre-miRNAs, other than the GGAG motif that aid in their interaction with LIN28 protein.

Publications

Original peer-reviewed articles

1. Khater S, Mohanty D* (2015) Deciphering the Molecular Basis of Functional Divergence in AMPylating Enzymes by Molecular Dynamics Simulations and Structure Guided Phylogeny. *Biochemistry* **54**:5209-5224.
2. Khater S, Mohanty D* (2015) *In silico* identification of AMPylating enzymes and study of their divergent evolution. *Sci Rep.* **5**:10804.
3. Khater S, Mohanty D* (2015) novPTMenzy: a database for enzymes involved in novel post-translational modifications. *Database (Oxford).* **2015**: bav039.

Review/Proceedings

1. Khater S, Anand S, Mohanty D* (2016) *In silico* methods for linking genes and secondary metabolites: The way forward. *Synt Sys Biotechnol* **1**:80-86.

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Chemical Glycobiology: Glycoform modulation, carbohydrate-based drug design, and glycomics

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Our research theme is the exploration of structural diversity and functional consequences of glycosylation in biological and immunological processes. Our objectives are design and development of (I) monosaccharide analogues for modulation of glycosylation that could potentially provide valuable inhibitors and disease modulators for auto-immune diseases and (II) novel carbohydrate-neuroactive (CH-NA) hybrid molecules in order to achieve metabolic glycan engineering (MGE) of the central nervous system (CNS) across the blood-brain barrier (BBB). Our approach might provide a vital access key to study the importance of glycosylation in CNS development and disorders.

***I. Glycoform modulation of monocyte/macrophage cells and inhibition of binding to E-selectin*.**

Our goal is to develop carbohydrate-based small molecule inhibitors of the biosynthesis of mucin-type *O*-glycans (MTOG), which participate in immune recognition, homing, and extravasation. Such small molecules would act as the first step in manipulating MTOG and may serve as valuable tools. Earlier studies from our laboratory have shown thiol-dependent inhibition of MTOG by Ac_5GalNTGc (**1**) via the GalNAc salvage pathway (**Fig. 1**) particularly on CD43 (leukosialin / sialophorin) (Agarwal, K., et al., *J. Am. Chem. Soc*. **135**, 14189-97 (2013)). Studies on the effect of GalNAc analogues on MTOG were performed on U937 (human histiocytic leukemia), K562 (human erythroleukemia), and THP-1 (human acute monocytic leukemia) cells using flow cytometry, lectin, and western blotting. Results showed consistent hypo-sialylation and reduction in sialo-glycoforms of CD43 upon treatment with **1**, but not with controls **2** and **3**. Treatment with the decoy inhibitor, **4**, showed results similar to **1**, but only to a moderate degree. Adhesion studies under static conditions were performed on protein-G

immobilized E-selectin coated surfaces using HL-60 cells pre-incubated with GalNAc analogues. Results showed that there was a 40 - 50 % reduction in the number of cells adhering after treatment with **1** (100 π M, 48 h), but not in controls suggesting the potential of such small molecules to modulate neutrophil endothelial cell interactions.

II. CH-NA hybrids for MGE across BBB.

Ability to modulate glycan structures in the brain across BBB would provide key access to study the importance of glycoconjugates in development and disorders of CNS in living animals. A panel of *N*-acyl-D-mannosamine

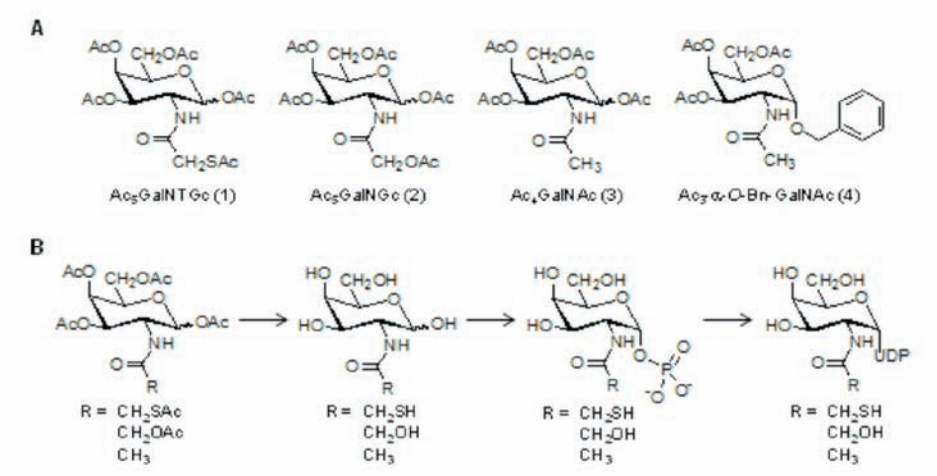

Figure 1. Interception of mucin type *O*-glycosylation pathway by analogues of *N*-acetyl-D-galactosamine (GalNAc). **A**. Chemical structures of synthetic GalNAc analogues employed in this study. **B**. Processing of analogues via the GalNAc salvage pathway resulting in the biosynthesis of UDP-GalNAc analogues which are then utilized by polypeptide:UDP-GalNAc transferases (ppGalNAcT, E. C. 2.4.1.41) for decoration on the extracellular domain Ser / Thr of polypeptides.

Mapping of mucin-type O-glycosylation sites on CD43 nano-LC-MS/MS.

In order to understand the structural diversity of various glycoforms of CD43, CD43-Fc-His was harvested from the supernatants of lentivirally transduced Jurkat cells, purified using metal affinity chromatography, and digested using trypsin and Glu-C. Through our HCD-PD-ETD nano-LC/MS/MS approach, we were able to identify 41 sites, including 32 new Ser / Thr as MTOG occupied sites. Taken together 57 sites out of predicted 80 90 MTOG sites on CD43 have been identified.

conjugated to carrier molecules were designed and synthesized. Treatment of SH-SY5Y (human neuroblastoma) cells with CH-NA hybrids resulted in inhibition of polysialic acid neural cell adhesion molecule (PSA-NCAM) by *N*-butanoyl but not *N*-acetyl analogues. Intravenous administration of CH-NA hybrids in mice showed a significant reduction in the brain PSA-NCAM levels when treated with *N*-butanoyl analogues. These results suggest the ability of CH-NA hybrids to modulate PSA-NCAM levels in living animals.

Kanwaljeet Kaur

Role of carbohydrates in modulating the structure and function of glycopeptides

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The project is aimed at understanding the role of carbohydrate domains in modulating the structure and function of glycopeptides by involving different model systems such as antimicrobial and thrombin-inhibitory peptides.

Objectives

1. Synthesis and structural characterization of glycosylated amino acids
2. Structure-function analysis of the synthetic glycoconjugates

Antimicrobial peptides

The effect of glycosylation on peptide structure and function depends on a variety of factors including glycan chain length. We have analyzed the effect of distal sugar and inter glycosidic linkage of disaccharides on the properties of Proline rich antimicrobial glycopeptides, formaecin I and drosocin. Their glycosylated analogs bearing lactose, maltose, and cellobiose, as a glycan side chain on their conserved threonine residue were synthesized earlier where these disaccharides possess identical proximal sugar and vary in the nature of distal sugar and/or inter glycosidic linkage. The comparative analyses of antibacterial activities of these analogs displayed that β-D-maltosyl-formaecin I and β-D-maltosyl-drosocin were more potent than that of respective β-D-Glc-, β-D-cellobiosyl- and β-D-lactosyl- analogs. Despite the differences in their antibacterial activity, all the analogs exhibited comparable binding affinity to DnaK. The comparative quantitative internalization studies of differentially active analogs revealed the differences in their uptake into bacterial cells.

Earlier we have reported the designing of the glycosylated analog of indolicidin and its non-glycosylated form which exhibited antibacterial activity similar to that of indolicidin against different bacterial strains. However, only

glycosylated analog was observed to be non-toxic towards eukaryotic cells. It was observed that the ability of these analogs to displace polymyxin B from LPS was comparable to that of native indolicidin. Designed analogs induced significant permeabilization of the inner membrane in *E. coli* similar to indolicidin. Bactericidal kinetic study results demonstrated that indolicidin and its analogs at 2XMIC killed the *E. coli* within 20 min.

Thrombin-inhibitory peptides

To gain a structural understanding of the observed biochemical effects of the designed bivalent HP6 thrombin inhibitors, the secondary structure studies by circular dichroism and *in-silico* studies were performed. The CD studies revealed that glycosylation does not alter the global secondary structure of the HP6 based analogs. *Ab initio* molecular docking studies of bivalent HP6 analogs revealed that as the glycine linker length increased, the intermediate fragment, which includes the sugar bearing Thr-43, progressively looped out and got distorted. Besides this, the side chain of Thr-43 was found to be oriented away from thrombin which may point out to a possible unfavorable region for glycosylation. Taken together, a distorted loop with an unfavorable region for bearing sugar explains the decreased affinity of glycosylated bivalent HP6 analogs.

To gain a structure based understanding of the observed kinetic behavior of differentially *O*-glycosylated variegin analogs, *in-silico* molecular docking studies were carried out. In order to accommodate within the torsional restraints allowed in docking protocol, the coordinates of tetrapeptide (HKTA) of variegin bearing the glycosylated threonine were taken. Docking studies revealed that sugars can indeed make favourable hydrogen bond interactions with thrombin. These observations explain the lower dissociation rate constants (k_d) of glycosylated analogs of variegin.

Publications

Original peer-reviewed articles

1. Lele DS, Dwivedi R, Kumari S, Kaur KJ (2015) Effect of distal sugar and interglycosidic linkage of disaccharides on the activity of antimicrobial glycopeptides. *J Pept Sci* **21**: 833-844.
2. Shabareesh PRV, Kaur KJ (2016) Structural and functional characterization of Hirudin P6 derived novel bivalent thrombin inhibitors-studying the effect of linker length and glycosylation on their function. *Chem Biol Drug Des* **88**:129-141.
3. Salunke DM, Khan T, Gaur V, Tapryal S, Kaur K (2016) Response to comment on three X-ray crystal structure papers. *J Immunol.* **196**:524-528.

Review/Proceedings

1. Shabareesh PRV, Kaur KJ (2015). Overview of the structural and functional impact of protein glycosylation in mediating protein protein interactions. *Trends Carbohydrate Res* **7**: 30-40.

Dinakar M. Salunke

Structure, interaction and design studies involving regulatory peptides and proteins

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Theme of research

The structural aspects of molecular recognition and its applications in analyzing the mechanisms associated with specific regulatory events and in rational molecular design.

Objectives

1. The structural aspects of molecular recognition and its applications in analyzing the mechanisms associated with specific regulatory events and in rational molecular design.
2. Understanding the protein architecture and the structural biology of various regulatory events.
3. Analysis of the structural principles of immune recognition and molecular mimicry.
4. Rational molecular design studies based on the above.

In order to address the issue whether degenerated antibodies can be a solution to neutralizing antibody problem against a fast mutating virus such as Influenza A virus, phage displayed human scFv libraries (Tomilinson's I and J) were screened against the peptide epitope of Influenza A virus as a physiologically relevant model system. Previously, screening of these libraries against the influenza A virus neutralizing epitope had provided substantial number of monoclonal phages showing binding to the epitope and to the selected analogues. Sequence analysis had shown that these clones have significant differences in their sequences at the CDR L2, L3, H2 and H3 regions and presence of amber stop codon in CDRH2 region. Cloning and expression of selected scFvs were standardized to get them in soluble form in amber suppressor strain, which resulted in poor protein yield (0.3-0.5 mg/ litre). Site directed mutagenesis was done in few scFvs to revert the

mutation from TAG to CAG and protein expression was checked in different expression strains to get the maximum yield. Protein was purified using affinity chromatography and improved protein yield of scFvs from 2 litres of culture media ranges between 2-3 mgs. Affinity measurement of the purified scFvs towards the peptide epitope as well its analogues was carried out using Surface Plasmon Resonance based assay. It was observed that most of the antibodies cross-reacted with more than one analogs of the neutralized epitope. Thus, the degenerate binding of the scFvs towards different variants indicates possible plasticity of antibody-antigen recognition even against the neutralizing epitopes of viruses that are known for immune evasion. In this library, DNA encoding millions of variable heavy (VH) and variable light (VL) chains linked by flexible glycine-serine linker products are cloned into a vector which is then engineered to express scFv fused to pIII minor capsid protein of filamentous bacteriophage of *E. coli*.

We observed that the cross-reactivity on both germline and affinity-matured antibodies is in defiance to the conventional rules of specificity. Crystallographic studies on antibody diversity have given newer insights into the mechanism of repertoire amplification at the germline as well as at the matured stage offering interesting physiological perspectives bringing out intriguingly new aspects of antigen recognition in humoral antibody response. These studies provide interesting insights into physiological processes without contradicting any rules of structural biology, the reason for the existence of dichotomy in specificity and degeneracy in the humoral response need to be addressed. With every new study, completely novel modes of binding and unexpected features continue to emerge from antigen-antibody complexes. Consolidation of these data in a coherent manner would help draw critical inferences that complete the picture and possibly provide answers for the observed shift in the paradigm in antigen specificity.

Publications

Original peer-reviewed articles

1. Jain A, Kumar A, Salunke DM (2016) Crystal structure of the vicilin from Solanum melongena reveals existence of different anionic ligands in structurally similar pockets. *Sci Rep.* **6**:23600.
2. Salunke DM, Khan T, Gaur V, Tapryal S, Kaur K (2016) Response to comment on three X-ray crystal structure papers. *J Immunol.* **196**:524-528.

Review/Proceedings

1. Kaur H, Salunke DM (2015) Antibody promiscuity: Understanding the paradigm shift in antigen recognition. *IUBMB Life*. **67**:498-505

Vidya Raghunathan

Biophysical and biochemical characterization of *Leishmania* phosphoglycerate kinase: An enzyme in the glycolytic pathway of parasitic protozoa

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Drug resistance in *Leishmania* is a menacing problem. Newer drugs and vaccines are necessary for treatment of Leishmaniasis due to various forms of the disease, toxicity of the current drugs and disease resistance in patients. The *Leishmania sp*. unlike mammalian counterparts uses multiple isoforms for many enzymes of the energy pathway, one of which is phosphoglycerate kinase or PGK. *Leishmania* PGK isoforms has some distinct structural features, as PGKB and PGKC differ primarily in the presence of a long extension at the C-terminus of PGKC. Drug development efforts can be targeted, either at the glycosome itself or at the enzymes present within them for which, targeting unique structural features is critical. We have used nuclear magnetic resonance spectroscopy and enzymology to study the structure of PGK isoforms. The metabolic profile of *Leishmania spp* cultures were mapped and correlated with the enzymological studies of the purified proteins.

Objectives

1. Expression, purification and determination of specific activities of PGKB-*Lmex* and PGKC-*Lmex* and steady state kinetics study. Comparison between PGKB-*Lmex* and PGKC-*Lmex* of, pH optimum of activity and enzyme inhibition by salt and suramin.

2. Conformational studies by theoretical and biochemical methods. ^{31}P NMR studies using substrate / enzyme (PGKB-*Lmex* or PGKC-*Lmex*) mixtures, with either no metal, or any one of $MgCl_2$, $CaCl_2$, $MnCl_2$ or $CoCl_2$, to determine the change in the dissociation constant of substrate with metal ions. Comparison with data from similar experiments in literature with yeast PGK using Mg-ADP and Mg-ATP.

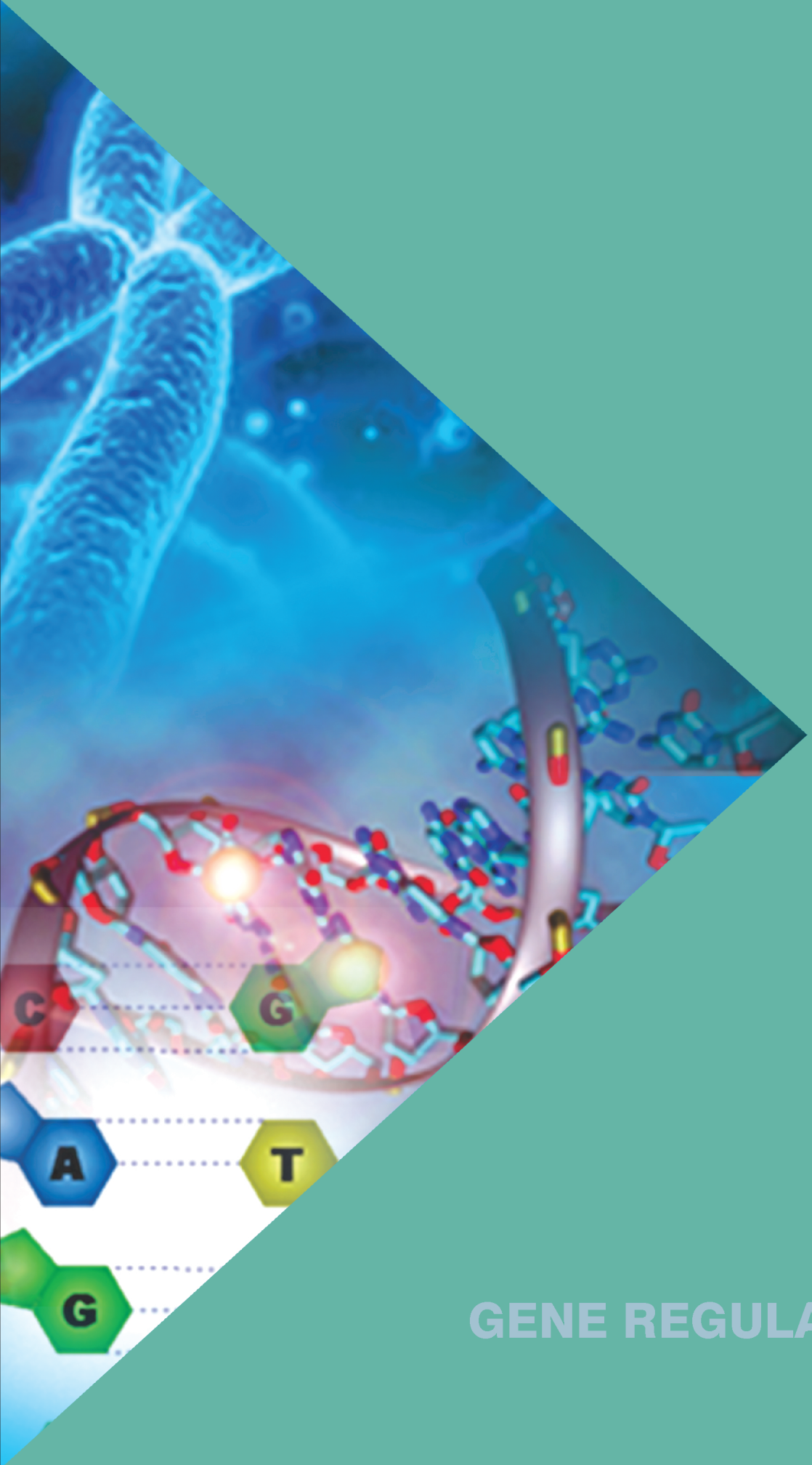
3. Peptide based studies of glycosomal membrane association of PGKC-*Lmex*. The peptides used in these studies will be evaluated as useful models to understand the structural basis of the biochemical differences between PGKC-*Lmex* and PGKB-*Lmex*.

4. Using promastigote and amasitoge cultures of *Leishmania spp* for metabolome mapping. The concentration of specific metabolites in the cell at a particular time can be monitored at the micromillimolar

level. The metabolites that can be detected are alanine, lactate, acetate, pyruvate, succinate, glycerol, urea, CO_2, oxalate, valine, glutamine and arginine.

Using homology based modeling to have a base structure for PGKC-*Lmex* and PGKB-*Lmex*. The softwares used were namely, SWISS-MODEL, ClusPro 2.0, Protein-Protein Docking, Jpred 4 and Chimera 1.10.1. The template used was of *T. brucei* phosphoglycerate kinase whose crystal structure in available in PDB. We also used biochemical data for various constraints in the calculations. Docking software is used to deduce the structural relationship between the core protein and the 63-mer extension (excluded in the template-based homology modeling). The resulting models are viewed in Pymol for analysis. Biochemical data on peptide-protein interaction in which, we have used synthetic peptides (same as that used in the work published in MBP,2012) mixtures with wild-type recombinant PGKB-*Lmex* narrowed down the conformational scape and give a model for whole PGKC-*Lmex*. Two models for PGKC-*Lmex* arise from the studies in both of which the 63-mer extension is available for interaction with the glycosomal membrane.

GENE REGULATION



GENE REGULATION

Sher Ali

Analysis of the Y chromosome linked genes and loci across different categories of Indian infertile males

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Human male infertility is caused owing to various factors including genes located on the Y chromosome. The infertility is defined as an inability of a couple to have a child and both male and female factors are equally accountable for it. Male infertility is often related to the spermatogenic failure. In spite of having normal spermiogram, a significant proportion of the males remain infertile. In the context of human male infertility, various genes on the human Y chromosome have been implicated besides hundreds of those on the autosomes. The azoospermic factors (*AZF*) i.e. *AZFa*, *AZFb*and *AZFc*and the genes located therein are known to be crucial for the maintenance of normal spermatogenesis. Besides, azoospermic factors, *SRY* gene, and DYZ1 arrays are reported to be affected in the blood DNA samples of infertile males having normal spermiogram. Mutations in and copy number variations of the *SRY* gene have been reported earlier in gonadal dysgenesis and sex reversal cases.

DYZ1 arrays showed copy number variations

The copies of DYZ1 arrays calculated using quantitative Real Time PCR (qPCR) were found to be significantly reduced in the infertile males. Statistical analysis of DYZ1 copies conducted by using one way ANOVA test in infertile males and normal fertile males showed normal distribution on the log scale. Upon applying the Bonferroni adjustment, copy number differences between the AZ and NM ($p = 0{\cdot}001$); OS and NM ($p = 0{\cdot}001$); INS and NM ($p = 0{\cdot}001$); INS and AZ ($p = 0{\cdot}001$); INS and OS ($p = 0{\cdot}004$) were found to be statistically significant. However, the same was non-significant in the OS and AZ ($p = 1{\cdot}000$) patients.

Copy number polymorphism of the *SRY,DAZ* and *BPY2*gene

The copy number analyses of *BPY2* and *DAZ* genes showed variation amongst the infertile males. About 11 OS, 21 AZ and 7 INS patients showed deletion of two copies of *DAZ* and one copy of *BPY2*. Two OS males showed deletion of all the four *DAZ* copies, while the remaining

patients had all the four copies of *DAZ* gene. The frequency of copy number variations (number of patients having corresponding *DAZ/BPY2* copies) across the OS, AZ and INS patients are shown in Fig. 1a, b and c. The intactness of *SRY* gene was confirmed using PCR. All OS and AZ males (Fig. 1a,b) had one copy of *SRY* (normal) except two patients (one OS and one AZ) who had 3 copies and less than one copy (0·33) of the *SRY,* respectively, suggesting mosaicism of the same in later. Among the INS, thirty six males showed normal *SRY* copies (Fig. 1c), while three showed two and one male had less than one copy (0·36, based on real time PCR data) of the *SRY* gene. *SRY* gene was localized onto the chromosomal metaphase and spermatozoa (Fig. 2).

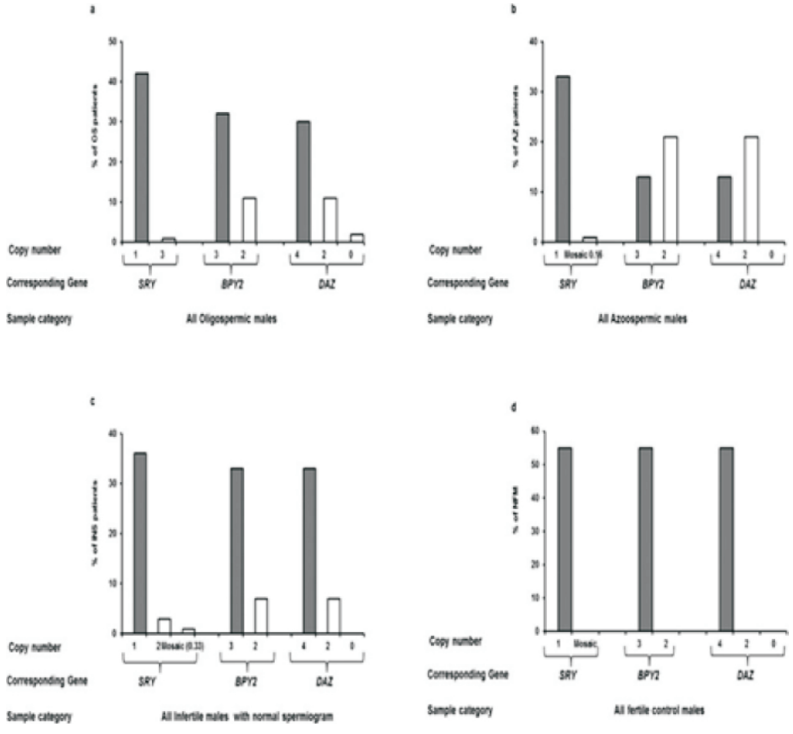

Figure 1. Copy number estimation of *SRY*, *BPY2* and *DAZ* genes using real time PCR.(**a-d**) Summarizes the distribution of copy number of *SRY*, *BPY2* and *DAZ* in oligospermic (**a**), Azoospermic(**b**), infertile males with normal spermiogram (**c**) and normal fertile males (**d**), respectively. X-axis depicts the gene of interest analyzed and the copy number assessed using real time PCR while the Y-axis indicates the % of males with corresponding copies for the genes. Grey bars indicate the normal copy number for the particular gene and the white bars indicate the copy number variation observed. A total of 43 OS, 34AZ and 40 INS patients were analyzed for copy numbers and each reaction was set in triplicates during real time PCR amplification.

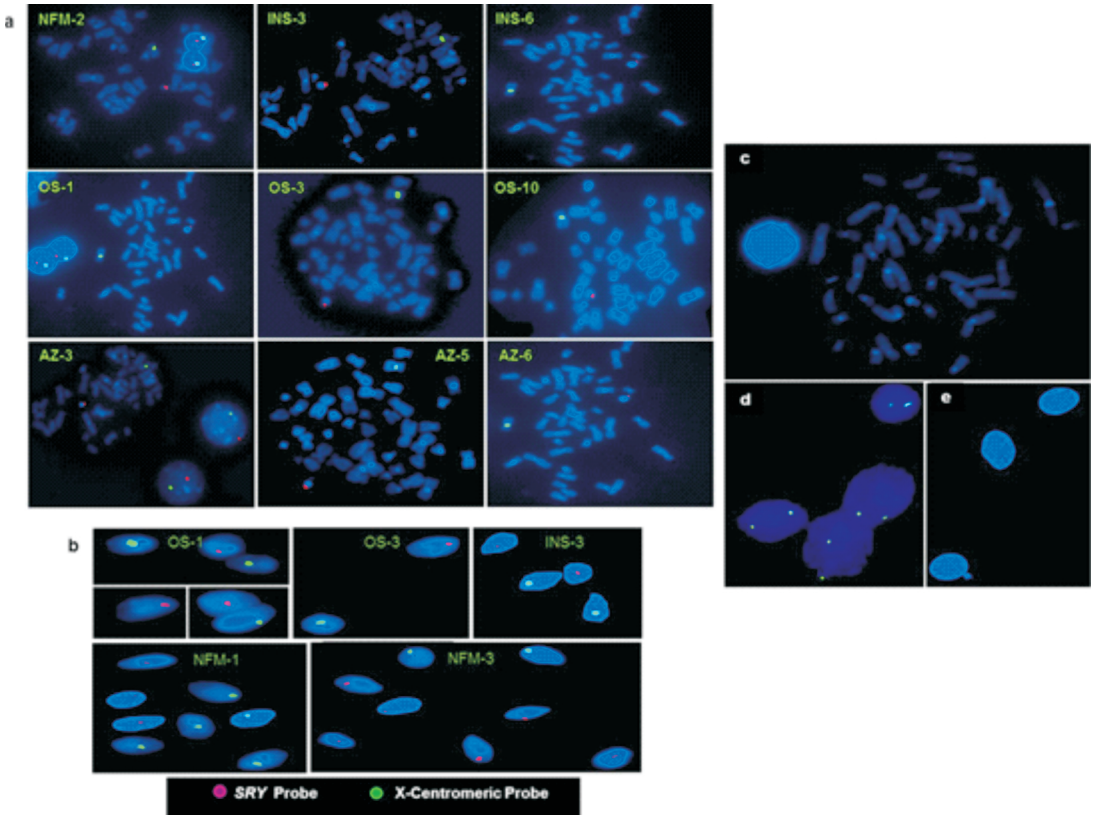

Figure 2. Chromosomal localization of the SRY gene on metaphases, interphase nuclei and spermatozoa using Fluorescence *in situ* hybridization (FISH). (**a**) The metaphase and interphase nuclei are stained withDAPI. The *SRY* gene localized on the Y chromosome is showing red signal and X-centromere, fluorescent green signal. (**b**) The figure represents a mapping of *SRY* gene on the individual sperm. All the Y bearing sperms are shown in red, whereas those of X-bearing ones are shown in green. (**c**) Male blood metaphase and (**e**)sperm samples were processed under identical conditions but not hybridized with the *SRY* probe to exclude the background signal. (**d**) Female sample hybridized with *SRY* probe. The spermatozoa are stained with DAPI. Patient IDs are shown in yellow. AZ indicates azoospermic; OS, oligospermic; INS, infertile males with normal spermiogram and NFM, normal fertile male.

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Original peer-reviewed articles

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Vinay Kumar Nandicoori

Deciphering the role of cell signalling in *M. tuberculosis* biology

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Serine/threonine protein phosphatase PstP of *M. tuberculosis* is necessary for accurate cell division and survival of pathogen

While there are 11 serine/threonine protein kinases in *M. tuberculosis*, only one cognate phosphatase, PstP, has been identified. Although PstP has been biochemically characterized and multiple *in vitro* substrates identified, its physiological role has not yet been elucidated. We have investigated the impact of PstP on cell growth and survival of the pathogen in the host. The PstP is the first gene of an operon that also carries rodA, pbpA, pknA and pknB genes. Previous studies have shown that overexpression of PknA or PknB in *M. smegmatis* or *M. bovis* BCG leads to altered cellular morphology. Our data indicates that the overexpression of PstP results in elongated cells, eventually resulting in compromised cell survival. Thus, it appears that the entire operon is involved in the modulation and maintenance

of cell morphology and cell division. To decipher the importance of PstP in regulating cellular events we generated *M. smegmatis* and *M. tuberculosis pstP* conditional mutant strains by carrying out gene replacements. The *M. smegmatis pstP* conditional mutant showed complete depletion of PstP in the presence of ATc. This depletion of PstP was detrimental to cell survival. Only partial depletion of PstP was observed in the *M. tuberculosis pstP* conditional mutant, but despite this, we observed ~one log-fold difference in cell survival, suggesting that stringent modulation of PstP expression is critical for optimal growth. PstP and nine of the eleven STPKs in *M. tuberculosis* contain a single transmembrane helix, connected to an extracytoplasmic domain. We observed that while membrane-anchoring of PstP is vital, the extracellular domain is dispensible for growth *in vitro*. Cell division in a complex process comprising cell elongation, septum formation and subsequent cytokinesis, involving a myriad of proteins. Scanning electron microscopy, flourescence microscopy and transmission electron microscopy experiments showed the formation of multiple septa upon PstP depletion (Figure 1). Thus, it appears that appropriate dephosphorylation of cell division proteins are critical to orderly cell elongation and cytokinesis. Data from mice infection experiments we have carried out indicate that even partial depletion of PstP compromises pathogen survival by ~10 fold. Interestingly partial depletion of PstP from an established infection (4 weeks post infection) also led to ~10 fold decrease in the survival, signifying the need for continued expression of PstP for the maintenance of *M. tuberculosis* infection. Taken together, our data suggests an important role for PstP in establishing and maintaining infection, possibly via the modulation of cell division events.

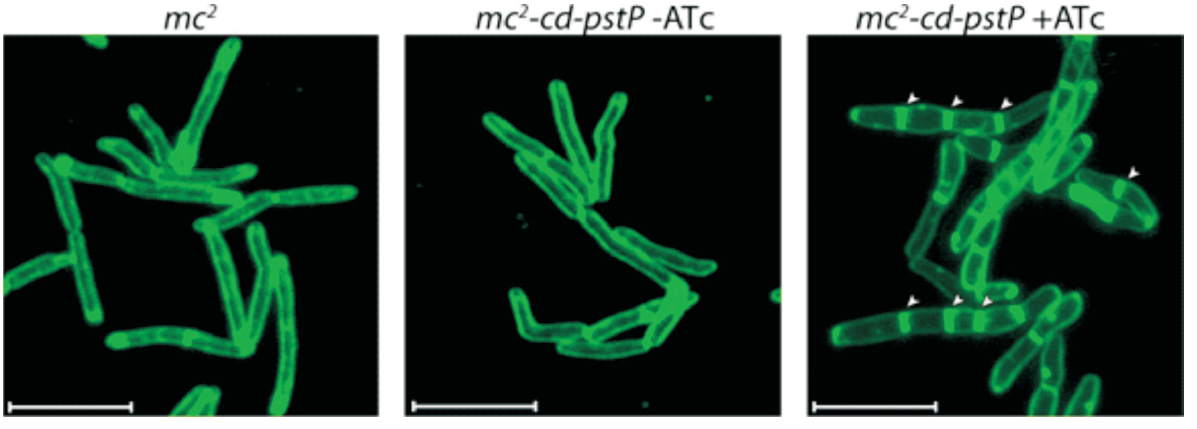

Figure 1: Flourescence microscopy analysis. FM64 labelling and microscopy of *mc²* and *mc²::cd-pstP* (conditional mutant) strains grown in absence or presence of ATc was performed as described in methods. Arrows indicate the presence of septa no arrows are visible to me. Scale bar: 5 µm

Depletion of *M. tuberculosis* GlmU from infected murine lungs effects the clearance of the pathogen

The mycobacterial cell envelope includes three layers of the cell membrane and a cell wall made up of peptidoglycan, mycolic acid, arabinogalactan and, lipoarabinomannan. Biosynthesis of the cell wall of bacteria is a complex process requiring enzymes localized to different cellular compartments. Due to the essentiality of the enzymes involved, they are

considered attractive targets for anti-microbial therapies. Most existing first line and second line drugs used to treat TB such as isoniazid, ethambutol, ethionamide and cycloserine, act on enzymes engaged in the synthesis of different cell wall components. UDP-GlcNAc is a critical metabolite both in prokaryotes and eukaryotes. In addition to the peptidoglycan synthesis, in gram negative bacteria UDP-GlcNAc is required for the synthesis Lipid A subunit of lipopolysaccharide and in gram positive bacteria it is required for Rha-GlcNAc linker, arabinogalactan, teichioc acid synthesis.

M. tuberculosis N-acetyl-glucosamine-1-phosphate uridyltransferase ($GlmU_{Mtb}$) is a bi-functional enzyme engaged in the synthesis of two metabolic intermediates N-acetylglucosamine-1-phosphate (GlcNAc-1-P) and UDP-GlcNAc, catalyzed by the C- and N-terminal domains respectively. While $glmU_{Mtb}$ was predicted to be an essential gene, till date the role of $GlmU_{Mtb}$ in modulating the *in vitro* growth of *M. tuberculosis* or its role in the survival of pathogen *ex vivo* / *in vivo* have not been deciphered. We found that absence of $GlmU_{Mtb}$ leads to extensive perturbation of bacterial morphology and substantial reduction in cell wall thickness under normoxic as well as hypoxic conditions. Complementation studies showed that the acetyl- and uridyl- transferase activities of $GlmU_{Mtb}$ are independently essential for bacterial survival *in vitro*, and $GlmU_{Mtb}$ is also found to be essential for mycobacterial survival in THP-1 cells as well as in guinea pigs. Depletion of $GlmU_{Mtb}$ from infected murine lungs, four weeks post infection, led to significant reduction in the bacillary load. $GlmU_{Mtb}$ and the acetyltransferase and uridyltransferase enzymes found in eukaryotes share very little sequence similarity. We have used shape based designing and developed a novel oxazolidine molecule, Oxa33, and characterized its ability to bind to the $GlmU_{Mtb}$ allosteric site. Further in order to determine the specificity of Oxa33, $GlmU_{Mtb}$ over expressing strains of *Rv* was used to determine the MIC. Both *in vitro* and *ex vivo* results (increased MIC or MBC) validate that Oxa33 specifically binds to $GlmU_{Mtb}$ inside the bacteria. Administrating the Oxa33 to fully infected (28 days) mice resulted in partial ablation of pathogen load in the lungs. Thus our study establishes $GlmU_{Mtb}$ as a strong candidate for intervention measures against established tuberculosis infection and Oxa33 can be pursued as a lead molecule, which needs to be developed further to improve its efficacy.

Publications

Original peer-reviewed articles

1. Suryadevara P, Yogeeswari P, Soni V, Devi, PB, Nandicoori VK, Sriram D (2016) Computational sampling and simulation based assessment of novel *Mycobacterium tuberculosis* glutamine synthetase inhibitors: Study involving structure based drug design and free energy perturbation. *Curr Top Med Chem* **16**:978-995.
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3. Yaseen I, Kaur P, Nandicoori VK, Khosla S (2015) Mycobacteria modulate host epigenetic machinery by Rv1988 methylation of a non-tail arginine of histone H3. *Nat Commun* **6:**8922.
4. Soni V, Suryadevara P, Dharmarajan S, Kumar S, OSDD Consortium, Nandicoori VK, Yogeeswari P (2015) Structure-based design of diverse inhibitors of *Mycobacterium tuberculosis* N-acetylglucosamine-1-phosphate uridyltransferase: combined molecular docking, dynamic simulation and biological activity. *J Mol Model* **21**:174.
5. Sharma G, Upadhyay S, Srilalitha M, Nandicoori VK, Khosla S (2015) The interaction of mycobacterial protein Rv2966c with host chromatin is mediated through non-CpG methylation and Histone H3/H4 binding. *Nucleic Acids Res.* **43:**3922-3937.

Patent

1. Nandicoori VK, Soni V, Yogeeswari P and Sriram D (2015) Depletion of *M. tuberculosis* GlmU from Infected Murine Lungs Effects the Clearance of the Pathogen *(Indian patent application 3167/DEL/2015 filed on 01/10/2015)*

Arnab Mukhopadhyay

Elucidating the molecular mechanisms of aging and innate immunity using *Caenorhabditis elegans* as a model system

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Using *Caenorhabditis elegans*, we are trying to decipher signalling events that culminate in alterations in gene expression during aging. We are trying to understand the complex interplay of transcription factors and co-regulators downstream of the Insulin-IGF-1-like signalling (IIS) pathway. Since Dietary Restriction (DR) is the only intervention that increases life span and delays age-onset diseases, we are trying to decode the molecular events that follow the initiation of DR.

A. Deciphering the coordinate regulation of genes downstream of the IIS pathway

We further characterized the role of ZFP-1/AF10 in life span regulation downstream of the IIS pathway. We found that the *C. elegans* gene encoding the mammalian AF10 interactor GAS41, *gfl-1* is also a direct target of DAF-16. We found that DAF-16, ZFP-1/GFL-1 and DAF-16 target genes form apparent feed-forward loops that control the amplitude and duration of target gene expression. We showed that ZFP-1 mediates this regulation by negatively influencing the recruitment of DAF-16/FOXO to their target promoters. By comparing the genome-wide binding patterns of DAF-16 and ZFP-1, we found that these proteins co-regulate a large number of genes. Consequently, *zfp-1* is required for the enhanced longevity observed on knockdown of IIS.

B. Role of alternative splicing in dietary restriction-mediated (DR) longevity

To study the complexity of gene regulation during DR, we performed transcriptomics analysis in a genetic model of DR, *eat-2(ad1116)*. Next, we devised a bioinformatics pipeline to compute differential alternative splicing of genes. We found that alternative splicing increases in the day 8 worms compared

to the day 1 worms. Interestingly, the *eat-2(ad1116)* worms showed higher alternative splicing at day 1 compared to WT of the same age. In order to identify splicing mediators those are required for alternative splicing during DR, we performed a targeted RNAi screen. One positive gene, *psm-15* suppressed *eat-2(ad1116)* life span without affecting WT life span. We knocked this gene down by RNAi in WT as well as in *eat-2(ad1116)* and found that *psm-15* controls a large portion of the alternative splicing events in *eat-2(ad1116)*.

C. Involvement of novel kinases in DR

We further characterized the requirement of p38 MAPK pathway in DR-mediated longevity. We found that the pathway is required for three different paradigms of DR in *C. elegans*. Genetic analysis showed that all the upstream kinases of the pathway are also required for DR-mediated life span. Interestingly, p38 MAPK pathway is not required for metabolic reprogramming that is associated with DR. However, it is required for upregulation of the xenobiotic detoxification genes.

To understand how *drl-2* affects life span, we performed transcriptomics analysis. We found that genes involved in xenobiotic detoxification are upregulated. Since we found that *drl-2* knock down worms have phenotypes similar to animals undergoing DR, we asked whether its life span extension is dependent on FA oxidation. To our surprise, we found that in the case of *drl-2* knockdown, life span is decoupled from metabolic reprogramming. Through genetic analysis, we confirmed that *drl-2* does not work through the IIS pathway. However, for it to extend life span, it needs suppression of *akt-1*.

D. Role of the Endoplasmic reticulum (ER) in DR-mediated longevity

We found that the transient upregulation of ER stress marker *hsp-4* as well as life span of DR animals is dependent on the IRE-1 and XBP-1, but not on ATF6/ATF-6 or PERK/PEK-1. Interestingly, when we mimicked the transient ER stress response that is characteristic of DR using ER stressor tunicamycin, we could increase life span of WT worms that were fed normally. Next, we used an *eat-2(ad1116);*$PolyQ_{40}$ strain and found that the age-dependent aggregation of $PolyQ_{40}$ is abrogated in a IRE-1-dependent manner. This indicates that the transient ER stress during DR may prime the organism to fold or eliminate proteins better. In this direction, we found that the ERAD components are upregulated during DR.

Publications

Original peer-reviewed articles

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2. Kumar N, Jain V, Singh A, Jagtap U, Verma S, Mukhopadhyay A (2015) Genome-wide endogenous DAF-16/FOXO recruitment dynamics during lowered insulin signalling in *C. elegans*. *Oncotarget* **6**:41418-41433.

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Molecular biology of infectious diseases

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Development of recombinant vaccine against beta toxin of *Clostridium perfringens:*

Beta toxin (btx), produced by *C. perfringens* types B and C, is the key antigen which is responsible for necrotizing enteritis and enterocolitis of domestic animals and has a significant economic impact on the agricultural industry worldwide. In order to generate a non-toxic mutants for a safer vaccine, and to understand the role of different residues in biological properties of the beta toxin such as binding to the host cell receptor, oligomerization and pore formation, a number of amino acid residues were targeted in different regions of the toxin. In order to identify the amino acid residues that might be important for the toxicity and other properties of beta toxin, multiple sequence alignment of beta toxin and other homologous pore forming toxins with known crystal structures was carried out. Based on multiple sequence alignment, four single residue mutation sites, and five deletion sequences were chosen. As the C-terminus of Beta toxin has been reported to be crucial for its toxicity, two deletion constructs of C-terminus were made to evaluate the possible role of these residues in the mechanism of action of the toxin. Deletion mutants and single residue mutations were generated by PCR and site-directed mutagenesis. The incorporation of intended mutations and deletions were confirmed by automated DNA sequencing. Beta toxin gene carrying different mutations and deletions were cloned, expressed and purified. All the mutant proteins expressed in insoluble fractions. The mutant proteins were tested for their cytotoxicity on HL-60 and THP-1 cells. One of the mutants was as toxic as the native toxin whereas some of the mutants were less toxic. Four mutants did not show any toxicity to HL-60 cells. These mutant proteins were subsequently used for immunization studies. Immunization of BALB/c mice with the fusion proteins generated a very good immune response with anti-sera of high titers. The anti-sera generated against all these mutant proteins were analyzed by Western blotting for the presence of toxin specific antibodies. Anti-sera generated by immunization with beta toxin mutants was tested for its neutralization ability using THP-1 and HL60 cells, and *in vivo* by recombinant beta

toxin challenge. Anti-sera generated against these proteins showed 60-100% protection *in vitro* and *in vivo*. The mutant proteins were also evaluated for their binding and oligomerization properties.

In order to develop epitope-based vaccine against beta toxin, four immunodominant epitopes of beta toxin identified using bioinformatics tools were expressed as fusion proteins with B-subunit of heat labile enterotoxin and the fusion proteins were characterized in detail. Immunization of BALB/c mice with the fusion proteins generated a very good immune response with anti-sera of high titers. None of the anti-sera was able to show any significant protection against beta toxin toxicity *in vitro* or *in vivo* except the anti-sera generated against the fusion protein LTB-$Epi_{140-156}$ which offered partial protection.

Publication

Original peer-reviewed article

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Madhulika Srivastava

Epigenetic regulation of the eukaryotic genome: Role of CTCF in organizing chromatin

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The mechanisms by which *cis*-acting regulatory elements interact with each other in the context of chromatin are incompletely understood even though such interactions are crucial for appropriate regulation of nuclear processes like transcription and VDJ recombination. CTCF is a DNA binding protein that can coordinate intrachromosomal and interchromosomal contacts and thus influence *cis*-DNA interactions in diverse ways. Antigen receptor loci present a useful framework to explore the nature of interactions amongst various regulatory elements. To accomplish VDJ recombination in a regulated manner, in addition to appropriate enhancer-promoter interactions, these loci also require physical interactions between RSS elements associated with the V, D and J segments located at large distances from each other on the chromosome. Higher order chromatin reorganization is necessary to bring them together prior to recombination. Since CTCF is an important contributing factor to long range interactions of chromatin, we are currently investigating the chromatin structure and organization of the wild type and genetically manipulated TCRβ loci to understand various aspects of CTCF based chromatin organization and its functional consequences.

As at other AgR loci, there are several CTCF binding sites (CBS) at the TCRβ locus. 3C assay was used to investigate the higher order chromatin organization defined by CTCF binding. The location of CBS and the observed interactions amongst them suggest that they could be important for maintaining the integrity of recombination center and/or facilitating locus contraction that precedes V-to-DJ recombination. Insertion of ectopic CTCF binding sites altered the chromatin loop scape. Consequently, the enhancer-promoter interactions necessary for the integrity of recombination center were influenced.

Ectopic CTCF binding could also potentially interfere in V-to-DJ recombination. Hence, we analysed the influence of the ectopic CTCF binding on the interactions pertinent for “locus contraction” that bring V segments in proximity of the recombination center. We examined the proximity of several V gene segments to the recombination center in wild type and mutant

alleles. Allele Specific 3C-qPCR analysis, was carried out in Rag1-deficient thymocytes isolated from mice that carried the wild type TCRβ allele or mutant allele (TCR-ins) that had the H19-ICR inserted and could bind CTCF ectopically. TCR-mut allele was an additional control allele that had insertion of H19-ICR incapable of binding CTCF.

Analysis of the interaction of Eβ with the promoters of V gene segments scattered through the locus by Allele specific 3C-qPCR analysis revealed that interaction of Eβ with each of the upstream V regions was reduced to about 50% in the TCR-ins allele compared to the wild type allele. Similar analysis was performed taking PDβ2-DJβ2 as an anchor for 3C analysis. Interaction of PDβ2-DJβ2 was also reduced to approximately 50% in TCR-ins allele. In each case, TCR-mut, unable to bind ectopic CTCF, resembled wild type TCRβ allele as expected. Since both Eβ and PDβ2-DJβ2 are a part of functional recombination center, it was not surprising that their proximity to the V segments was reduced to a similar extent. Strikingly, the extent of reduction was the same as observed earlier for the interactions amongst CTCF binding sites. These data suggest that perturbations in CTCF dependent interactions are reflected in the interactions of the recombination center to the V segments leading to functional alterations in the choice of V segments for V-to-DJ recombination.

Our data suggest that the ectopically bound CTCF in TCR-ins allele competes with the endogenous CTCF binding sites of the TCRβ locus for interactions and hence reduces the interactions amongst various regions to approximately 50%. This is an intriguing observation since its ability to compete with other CTCF binding sites should not preclude the access of the recombination center to the upstream V segments. Additionally, while the reduction in the interaction of V segments to the recombination center was approximately 50%, the use of the upstream V regions for V-to-DJ recombination was reduced dramatically to 10%-20%. This suggests that not just overall proximity of the recombining segments *via* locus contraction but also the intricate configuration of the chromatin loop is critical for successful recombination which might have been altered by the ectopic CTCF in the TCR-ins allele.

Further, the location of CTCF binding sites on TCRβ locus is very interesting. The two sites that flank the recombination center might be responsible for defining the Eβ regulated domain. We have initiated an investigation to examine the ability of the CTCF binding sites to act as insulators using *in vitro* enhancer blocking assays.

Publication

Original peer-reviewed article

1. Varma G*, Rawat P*, Jalan M, Vinayak M, Srivastava M (2015) Influence of a CTCF dependent insulator on multiple aspects of enhancer mediated chromatin organization. *Mol Cell Biol* **35**: 3504-3516.

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Role of cell signaling in eukaryotic development

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We are interested in the dissection of signaling and trafficking mechanisms that operate in diverse cell types. Here, is a brief description of our recent studies:

I. Dissection of intracellular signaling and trafficking cascades of *Plasmodium falciparum*.

a. cAMP and calcium signaling in the blood stage development of malaria parasite.

As reported last year, we have elucidated the role of PfCDPK1, which is an effector of calcium signalling, in host RBC invasion by *P. falciparum*. This was achieved by using a parasite line in which PfCDPK1 expression was knocked down by tagging it to FKBP Death Domain (DD). Further studies indicated that PfCDPK1 regulates the attachment of merozoites to RBC, which is a key step in invasion mediated by proteins secreted from micronemes. *Plasmodium* utilizes both sialic acid dependent and independent pathways for RBC invasion and secretes different sets of ligands for this purpose. PfCDPK1 knock down mainly impaired the neuraminidase sensitive invasion. Quantitative proteomics studies revealed that PfCDPK1 may target diverse classes of proteins including proteins involved in the invasion. Several Inner Membrane Complex proteins like GAP45, IMC1c and IMC1g were found to be differentially phosphorylated. The components of the low molecular weight rhoptry complex RAP1 and RAMA were also differentially

phosphorylated. PfCDPK1 interacts with RAP1 and RAP2 and phosphorylates RAMA. It is possible that it may have an ancillary role in post-invasion events like PVM formation in which these proteins are implicated.

b. Role of phosphoinositides in parasite signaling and trafficking

We have extended our interest in PIP-mediated signaling and trafficking by understanding the function of CDPK7, which interacts with PI(4,5)P2 in both *Plasmodium falciparum* and *Toxoplasma gondii.* A significant defect in phosphatidylcholine metabolism was found in PfCDPK7-KO parasites. These studies unraveled a novel role of CDPK7 in phospholipid metabolism/trafficking in Apicomplexan parasites.

II. Molecular mechanisms that regulate Cell Cycle Related Neuronal Apoptosis (CRNA)

p53 family member p73 has been implicated in neuronal differentiation and death. TAp73 isoforms interact with a p53 binding site on miR-34a promoter and regulates its expression. We specifically evaluated the role of TAp73 in miR-34a biogenesis and CRNA by overexpressing TAp73α. miR-34a promoter activity, which was inhibited in response to Aβ_{42}, was restored upon TAp73α overexpression. A time course experiment revealed that a slight increase in TAp73 protein levels was followed by a steep decline after 24-48h of treatment. A similar decrease in TAp73 was observed in neurons of APP/PS1 Tg AD mice and was significantly lower in the >12m old APP/PS1 Tg mice. The decrease in TAp73 expression corroborated well with the loss of miR-34a in Aβ_{42}-treated neurons or neurons from APP/PS1 Tg mice. The loss of TAp73 and miR34a upon Aβ_{42} treatment was significantly reverted by proteasomal inhibitor MG132 indicating that Aβ_{42} promotes proteasomal degradation of TAp73. Further studies suggested that aberrant and sustained MEK-ERK pathway activation promotes TAp73 degradation resulting in attenuated miR-34a expression.

Collectively, these and other results strongly indicated that TAp73 may be a key player in the prevention of CRNA as it regulates miR-34a expression.

Publication

Original peer-reviewed article

1. Modi PK, Jaiswal S, Sharma P* (2015) Regulation of neuronal cell cycle and apoptosis by microRNA-34a. *Mol Cell Biol* **36**:84-94.

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Rajesh S. Gokhale

Reconstructing the chemico-cellular trestle to decipher biology of tuberculosis and vitiligo

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In order to understand the pathogenesis of tuberculosis, we focused towards the complex cell envelope coat of mycobacterium - a unique feature of this pathogen. Our research has provided a new insights into the chemico-cellular trestle of mycobacteria, providing a new opportunity to delineate remarkable dormancy dynamics of this infectious agent. The commensal bacteria constitute an important component of the resident microbiome and are intricately linked to skin health. Recent studies describe an association between altered skin microbial community and epidemiology of diseases, like psoriasis, atopic dermatitis etc. In order to understand the association of localized alterations in Vitiligo skin with cutaneous microbiota, we have explored the microbial community profiles of the lesional and non-lesional skin patches of vitiligo subjects using 16S rRNA phylotyping. Our study reveals dysbiosis in the diversity of microbial community structure in lesional skin of vitiligo subjects. Although the individual specific signature is dominant over the vitiligo-specific microbiota, we noted a clear decrease in taxonomic richness and evenness in lesional patches. Investigation of community specific correlation networks reveals a distinctive pattern of interactions between resident bacterial populations of the two sites (lesional and non-lesional). While Proteobacterial species constitute the central regulatory nodes (with respect to the degree of interaction) in the non-lesional skin, species belonging to Firmicutes dominate on lesional sites. A key aspect of our study is analysis between matched sites from the same individual. This eliminates a likelihood of variations arising from interpersonal differences and adds robustness to the analysis. This is the first study of microbial analysis from vitiliginous subjects and provides insights into the alterations and adaptations of bacterial networks in the diseased state. We propose that the changes in taxonomic characteristics of vitiligo lesions could play a crucial role in altering the maintenance and severity of the disease. This study aids in understanding the potential role of microbial community in vitiligo pathophysiology and could be of potential therapeutic and

diagnostic significance. We have also set up a major programme in the area of understanding skin pigmentation homeostasis, with the particular interest in delineating chronic unpredictable disfiguring disorder vitiligo.

Publications

Original peer-reviewed articles

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3. Srivastava S, Chaudhary S, Thukral L, Shi C, Gupta RD, Gupta R, Priyadarshan K, Vats A, Haque AS, Sankaranarayanan R, Natarajan VT, Sharma R, Aldrich CC, Gokhale RS (2015). Unsaturated lipid assimilation by mycobacteria requires auxiliary cis-trans Enoyl CoA Isomerase. *Chem Biol* **22**:1577-1587.
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5. Anand A, Verma P, Singh AK, Kaushik S, Pandey R, Shi C, Kaur H, Chawla M, Elechalawar CK, Kumar D, Yang Y, Bhavesh NS, Banerjee R, Dash D, Singh A, Natarajan VT, Ojha AK, Aldrich CC, Gokhale RS (2015). Polyketide quinones are alternate intermediate electron carriers during mycobacterial respiration in oxygen-deficient niches. *Mol Cell* **60**:637-650.
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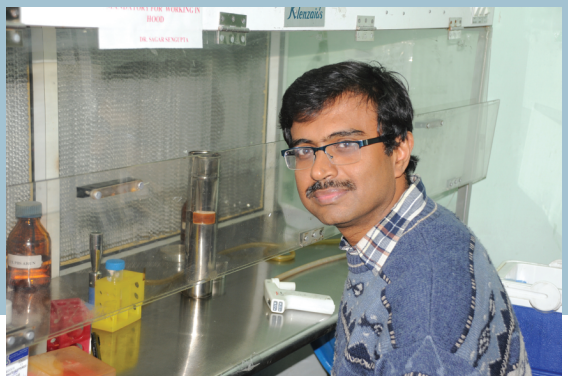

Sagar Sengupta

Determining the signaling and repair pathways that are altered in human cancer

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Phosphorylation of BLM at Thr182 is essential for the maintenance of genome stability

Lack of BLM turnover during mitosis may adversely affect its functions during the maintenance of chromosomal stability during mitosis. As a first step, we studied whether the lack of Thr182 phosphorylation affected BLM localization onto the Ultra Fine Bridges (UFBs). The total number of PICH stained UFBs per cell were similar in both wildtype and T182A expressing cells, indicating that there was no difference in the extent of UFB formation. However in 50.1 cells (i.e in cells in which BLM is not phosphorylated at Thr182), BLM failed to optimally localize to the UFBs especially after sub-optimal aphidicolin treatment. Inhibition of BLM degradation when Fbw7 expression was ablated by siRNA or by treatment with CDK2/Cyclin A2 inhibitor, roscovitine led to increased BLM accumulation onto the UFBs in only wildtype BLM expressing 4.3 cells. This occurs, as the Chk1/Chk2 dependent Thr182 phosphorylation was functional in the 4.3 cells lacking Fbw7 or post-roscovitine treatment. Consequently inhibition of Thr182 phosphorylation when 4.3 cells were grown in

the presence of both Chk1/Chk2 inhibitors led to an almost complete lack of BLM accumulation on the UFBs. The lack of Thr182 phosphorylation on BLM was the reason due to which frequency of the UFBs did not increase in 50.1 cells even in the absence of Fbw7. The lack of BLM localization to the UFBs led to imperfect mitotic segregation, causing increased duration of mitosis in 50.1 cells, culminating in their longer doubling time. Interestingly 50.1 cells expressing Thr182Ala BLM mutant show a high level of lagging chromatin and micronuclei formation. The greater number of 53BP1 foci was observed in 50.1 cells, indicating the higher levels of endogenous DNA damage. The 50.1 cells also had a significant increase in the number of breaks and quadri radials. Hence the lack of BLM Thr182 phosphorylation leads to chromosomal instability phenotypes, which are the characteristic features of the BS patients.

Lack of localization of RECQL4 to mitochondria is essential for the prevention of aerobic glycolysis dependent cell invasion

Recent results from the lab led to the question whether the lack of mitochondrial localization of RECQL4 in RTS patients affected the functioning of the mitochondria and thereby contributed to the neoplastic transformation process. To answer this question, a statistically significant reduction in the mtDNA copy number was observed in the RTS patient fibroblasts. This decrease in mtDNA copy number was due to the absence of mitochondrial localization of RECQL4 as cells expressing RECQL4 (Δ84) also showed a similar decrease. The mitochondrial mass was also found to be decreased in RTS patient fibroblasts and RECQL4 (Δ84) cells. These changes in the mitochondrial integrity were reflected in altered mitochondrial ultrastructure, whereby in contrast to GM07532, fibroblasts from RTS patients showed aberrant mitochondrial morphology, distorted cristae resulting in reduced cristae surface. Activity assays carried out using the isogenic cell lines indicated that the relative activity of either Complex I or Complex IV was not altered irrespective of the status of RECQL4. In contrast, the activity of F_1F_0-ATP synthase (Complex V) was diminished in AG05013 cells. Restoring the expression of wild type RECQL4, but not RECQL4 (Δ84), reinstated Complex V activity. Consequently, the levels of intracellular ATP were more in GM07532 and RECQL4 (WT) cells compared to AG05013 and RECQL4 (Δ84) cells. This difference in the levels of intracellular ATP was abolished in cells grown in the presence of F_1F_0-ATP synthase inhibitor, oligomycin. In concordance with the results indicating gross mitochondrial dysfunction, membrane potential was reduced in RTS fibroblasts and in AG05013 cells expressing either AcGFP or AcGFP RECQL4 (Δ84). Consequently, mitochondrial ROS was increased in RTS patient cells and in cells lacking mitochondrial RECQL4.

The high levels of mitochondrial ROS in cells lacking mitochondrial RECQL4 led to the expectation of very low levels of SOD2 in these cells. In contrast, cells lacking mitochondrial RECQL4 express increased SOD2 levels and thereby showed lower SOD2/AcLys68 SOD2 ratios compared to cells expressing wildtype RECQL4. This indicates that the high level of SOD2 in cells lacking mitochondrial RECQL4 was catalytically inactive. SIRT3 deacetylates and thereby activates SOD2 which in turn scavenges the mitochondrial ROS. Reduction in SIRT3 activity was observed in cells lacking mitochondrial RECQL4. Thus decreased SIRT3 activity possibly caused inactive SOD2 to accumulate.

The above results indicated cells lacking mitochondrial RECQL4 may utilize the inefficient but faster aerobic glycolysis process to generate ATP viaglycolytic. Indeed in contrast to GM07532, all tested RTS patient cells had significantly increased glucose uptake, due to

which activity of the glycolytic enzyme 6-Phosphofructokinase was enhanced in cells not expressing RECQL4. Consequently, the excess of the glucose consumed is converted to lactate instead of pyruvate, thereby causing an increase in the lactate to pyruvate ratio in cells lacking the mitochondrial localization of RECQL4.

The augmented glucose uptake, the increased PFK activity and the accumulation of lactate in cells lacking mitochondrial RECQL4 should lead to the acidification of the cellular milieu, thereby providing a micro-environment conducive for the greater invasive capability of cells. To test this hypothesis, *in vitro* matrigel invasion assays were carried out. Cells from RTS patients and those expressing RECQL4 (Δ84) have increased invasive capability. Thus using isogenic cell lines in conjunction with immortalized RTS patient fibroblasts, we have determined that alterations in the mitochondrial bioenergetics occur in the absence of the localization of RECQL4 to the organelle. This may contribute towards the neoplastic transformation process, commonly observed in RTS patients.

Publications

Original peer-reviewed articles

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2. Kharat SS, Tripathi V, Damodaran AP, Priyadarshini R, Chandra S, Tikoo S, Nandhakumar R, Srivastava V, Priya S, Hussain M, Kaur S, Fishman JB, Sengupta S* (2016). Mitotic phosphorylation of Bloom helicase at Thr182 is required for its proteasomal degradation and maintenance of chromosomal stability. *Oncogene* **35**: 1025-1038.

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Patent

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Sandeep Saxena

Understanding the regulation of DNA replication

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DNA replication is a vital process of life and must be completed precisely during each cell cycle. When the mammalian cell experiences DNA damage, it activates checkpoint mechanisms to stall the progression of cell cycle and DNA replication. Our laboratory is working towards understanding the mechanisms by which microRNAs and checkpoint proteins stall the cell cycle, preventing genomic instability and cancer.

We are studying the regulation of replication machinery during stress in order to identify underlying mechanisms responsible for inhibition of essential replication factors during stress. We are trying to understand the role of ubiquitination machinery in regulating replication proteins under normal and stressed conditions. Further, we are investigating the cellular response to aberrations in replication complexes. The objective is to identify yet unknown checkpoint pathways that monitor the replication apparatus. Emerging evidence suggest that microRNAs target genes that regulate DNA replication and cell cycle progression and our aim is to determine the role of microRNA in regulating the DNA replication machinery as the cell progresses from one phase to the next. This would provide an insight into the mechanisms by which microRNAs regulate mammalian cell cycle and DNA replication during normal and pathological conditions. We are trying to understand the role of replication proteins in centrosomal stability. Summing up, we are attempting to unravel the protective regulatory control of mammalian cells, failure of which is likely to cause genomic instability.

GINS is required for centrosome integrity during mitosis

In eukaryotes, the tetrameric GINS complex (comprising of Sld5, Psf1, Psf2 and Psf3) is involved in both initiation and elongation stages of DNA replication. We report that GINS is essential for resisting the forces that converge on centrosomes during chromosome congression. GINS localizes to the centrosomes and in its absence, the structural integrity of centrosomes is lost leading to centriole splitting as well as pericentriolar material fragmentation. GINS-deficient cells form multipolar spindles with disengaged centrioles, resulting in an aberrant spindle apparatus. We

observed that in the absence of GINS, centrosomes are unable to endure these microtubule-mediated forces leading to spindle pole fragmentation. Thus, GINS has an as yet unknown function of ensuring spindle pole resistance to traction forces exerted during chromosome congression.

Figure 1

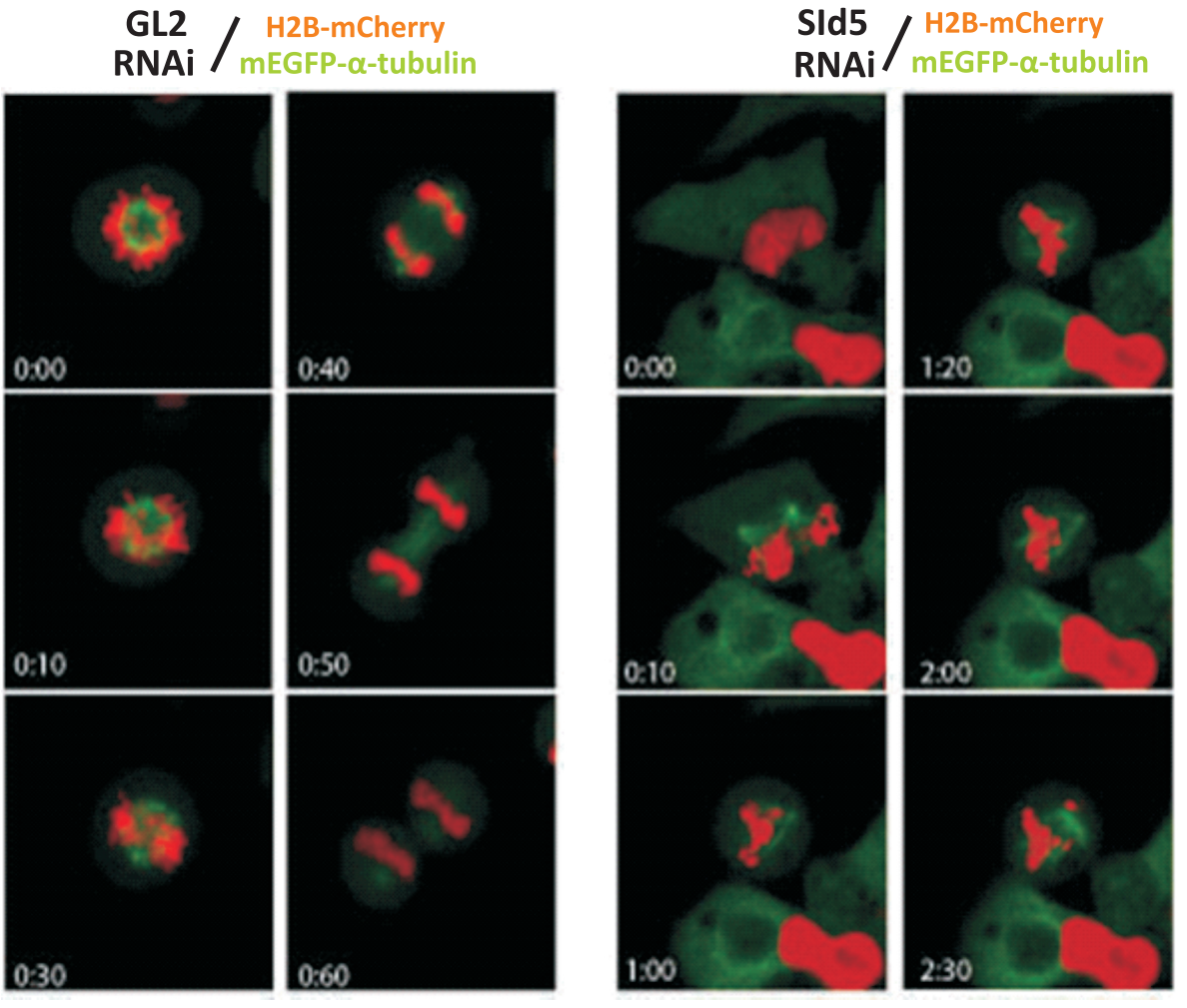

Figure 1. GINS depletion does not cause centrosome over-duplication in interphase. HeLa cells stably co-expressing a red chromatin marker (core histone H2B fused to monomeric cherry; H2B-mCherry) and a marker for microtubules (mEGFP-α-tubulin) were transfected on three consecutive days with control *GL2* or *GINS* siRNA followed by live-cell imaging for almost 4 h.

Role of alternate single-stranded DNA-binding proteins in checkpoint signaling

We have discovered a novel pathway for checkpoint activation: we propose that ATR-ATRIP can bind to DNA by two mechanisms: the first is RPA-dependent and the second requires the hSSB1/2-INTS3 complex. We propose that in case the RPA-dependent complex is non-functional, the alternate complex may serve to activate the checkpoint during genomic stress.

Figure 2

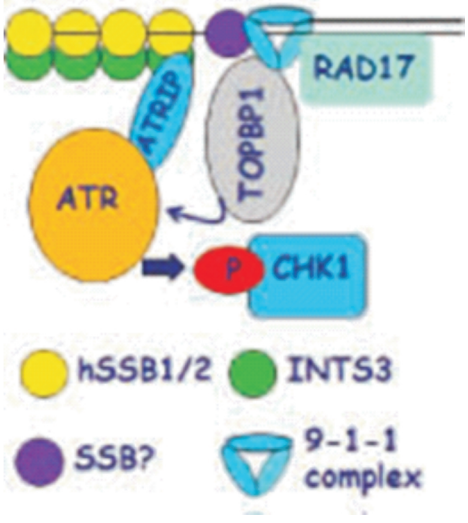

Figure 2. A model for ATR-ATRIP recruitment by the hSSB1/2-INTS3 complex. Single-stranded DNA (ssDNA) generated at the sites of genomic stress is coated by the hSSB1/2-INTS3 complex in the absence of RPA. The N-terminus of INTS3 associates with the oligonucleotide/oligosaccharide-binding fold of hSSB1/2, which binds to the ssDNA. ATR-ATRIP complex is then recruited to the hSSB1/2-INTS3 bound ssDNA.

In the future, we would determine the role of Chk1 in RPA70 depleted cells. Also, we would ascertain if microtubular forces contribute to the mitotic aberrations observed after GINS depletion. We would determine the role of specific kinesin family members by co-depleting them with GINS. Thus, we would try to understand the mechanism of GINS in maintaining centrosomal integrity. We are trying to understand how microRNAs regulate mammalian cell cycle and DNA replication in normal and pathological conditions. In future, we would determine if microRNAs targeting the same cell cycle genes alter the activity of their targets in different physiological states.

Publication

Original peer-reviewed article

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Sanjeev Das

The role of tumor suppressors in stress response

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The focus of the lab is to understand the function and regulation of tumor suppressors. Here, we report the work carried out on one such protein viz. sirtuin 6 (SIRT6). Of the mammalian sirtuins, SIRT6 recapitulates many of the biological functions of the founder member of the sirtuin family, yeast Sir2. At the molecular level, SIRT6 regulates the expression of a large number of stress-responsive and metabolism related genes, promotes genomic stability and DNA repair. To understand the complexity of SIRT6 interactome, we also carried out a LC-MS/MS based proteomics screen. The result of LC-MS/MS screen identified several novel interacting proteins including Pyruvate Kinase M2 and Ubiquitin-protein ligase E3A (UBE3A).

Pyruvate kinase catalyzes the final rate-limiting step of glycolysis. Most cancer cells express high levels of PKM2 as it promotes aerobic glycolysis and provides a selective advantage for tumor formation. Thus, PKM2 is reported to be upregulated in a wide range of cancers. In addition to its well-characterized cytosolic functions, several studies have reported the nuclear localization of PKM2 in response to different signals. In the nucleus, PKM2 functions as a transcriptional coactivator and protein kinase to trigger the expression of various genes thereby bestowing cancer cells with survival and growth advantage. However, the molecular mechanisms underlying the dynamic regulation of PKM2 nuclear localization are poorly understood. Our results indicated that SIRT6 binds to and deacetylates nuclear PKM2 at lysine 433 residue. SIRT6-mediated deacetylation results in PKM2 nuclear export. We have further identified exportin 4 as the specific transporter mediating PKM2 nuclear export. As a result of SIRT6-mediated deacetylation, PKM2 nuclear protein kinase, and transcriptional coactivator functions are abolished. Thus SIRT6 suppresses PKM2 oncogenic functions resulting in reduced cell proliferation, migration potential, and invasiveness. Furthermore, studies in mouse tumor models demonstrate that PKM2 deacetylation is integral to SIRT6-mediated tumor suppression and inhibition of metastasis. Additionally, reduced SIRT6 levels correlate

with elevated nuclear acetylated PKM2 levels in increasing grades of hepatocellular carcinoma. These findings provide new insights into the pivotal role of deacetylase activity in SIRT6 tumor suppressor functions.

UBE3A functions as an E3 ligase in the ubiquitin proteasome pathway. UBE3A was originally discovered to ubiquitylate and promote degradation of tumor suppressor p53, through which it plays a pathogenic role in human papillomavirus-induced cervical cancer. Since our previous findings suggested that SIRT6 is regulated by ubiquitin proteasome system, we hypothesized that UBE3A could be the cognate E3 ligase regulating SIRT6 the protein levels. To further investigate, we examined whether UBE3A physically interacts with SIRT6. Our results indicated that HECT domain of UBE3A binds to the catalytic domain of SIRT6. We further observed that UBE3A negatively regulates SIRT6 at protein level. *In vivo* ubiquitylation assays indicated that SIRT6 undergoes extensive ubiquitylation only in unstressed conditions and not upon metabolic stress. Notably, no ubiquitylated SIRT6 was detected in the absence of UBE3A which confirmed that it is the major E3 ligase regulating SIRT6 levels. Additional studies revealed that SIRT6 gets polyubiquitylated in a K48-linked manner. These results suggest that UBE3A is a key determinant of SIRT6 levels. Further studies are being carried out to understand the physiological significance of UBE3Amediated SIRT6 ubiquitylation.

Publications

Original peer-reviewed articles

1. Bhardwaj A, Das S (2016) SIRT6 deacetylates PKM2 to suppress its nuclear localization and oncogenic functions. *Proc Natl Acad Sci USA* **113**: *E538-E547*.
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Vijay K. Yadav

Molecular and genetic identification of physiological pathways that regulates bone physiology and their therapeutic implications

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Skeleton in vertebrates serves multiple mechanical, hematopoietic, and endocrine functions. In order to perform its functions properly, skeleton continuously renews itself through a homeostatic process known as bone remodeling - a process carried out by osteoblasts and osteoclasts to maintain a fine balance between bone formation and resorption. An imbalance in this process leads to skeletal diseases, such as osteoporosis. Osteoporosis (OP) is a multifactorial disease involving interactions within bone, interactions between bone and other organ systems and influence of environment. Osteoporosis is caused by a deregulation in these interactions affecting the process of bone remodeling where in degradation of bone carried out by osteoclasts exceeds formation of new bone carried out by osteoblasts. Although 3 out of 5 females face osteoporosis after menopause and 2 out of 5 males face osteoporosis after 50 years of age the pathophysiology of this disease remains poorly understood. The paucity of basic knowledge surrounding OP is reflected in the clinic as there exist only one therapy that can increase bone formation and cure osteoporosis till date i.e., intermittent injections of parathyroid hormone (PTH). However, PTH cannot be given for more than 2 years due to side effects, needs to be injected daily, and is costly. The need of an hour is therefore to identify therapies that can increase bone formation and cure osteoporosis in humans. We have recently shown that stomach-expressed protein Gif regulates vitamin B_{12} absorption that in turn regulates the liver synthesis of taurine, a powerful regulator of bone formation. Currently, investigations are underway to investigate the mechanism(s) through which this novel stomachliver- bone endocrine axis regulates bone mass.

ANCILLARY RESEARCH

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Production of transgenic and other animal models for biomedical research

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We extend collaborative help in using specific animal models (transgenic or non-transgenic) for biomedical research. This service is provided by NII to various laboratories of the nation. Task of making various transgenic animals for other investigators was undertaken as and when the constructs were provided. Fore-founder animals were given to P.I.'s for generating transgenic lines to address their respective scientific goals. Work is being continued and major findings usually lead to publications.

We are also working to develop new easier techniques for making transgenic animals, using genes relevant to human health and diseases. One of the objective is to develop easy techniques for making large animals expressing therapeutic products in their milk for increasing affordability of such therapeutics, by masses. In the past, testicular transgenesis was successfully established for *rats* for overexpressing genes. Following this new procedure, we have succeeded in interrupting expression of specific genes *in vivo* by use of shRNA in transgenic rats. We established this method of generating knock down rats through testicular gene integration. Analysis of GFP expression in F_1 generation of shRNA knockdown rat by IHC confirmed for germline transmission. Slot blot analysis confirmed for genomic integration of shRNA vector in the progeny. The relative percentage of gene expression in the testis of shRNA knockdown rat lines showed that there was a significant decrease in the mRNA levels of corresponding gene in the respective knock down rat with respect to their expression in the control - lacz knockdown rat. We targeted different testicular

genes (Eaf2, Ninj2, Nmu, and Nr4A3) with different degree of knock down (19% in Eaf2 rat, 30% in Ninj2 rat, 66% in Nmu rat, 47% in Nr4A3) for shRNA mediated knock down. To study the cell specific down regulation of target mRNA, Ninj2 was expressed under control of Sertoli cell specific pomoter. Evaluation of the role of Ninj2 knock down on the reproductive parameters of transgenic rat revealed a small but significant change in the testis weight and sperm count in the transgenic rat as compared to that of control. Histological sections of testis from 10 months old transgenic rat showed a significant number of degenerated seminiferous tubules along with some normal tubules. However, the degenerated tubules were nearly absent in the age matched wild type control sections. Therefore, we concluded that even 30% Ninj2 knock down in Sc of pubertal rats can inhibit spermatogenesis quantitatively. The shRNA knock down rat generated by this method provided an ideal model to study the biological function of these genes in spermatogenesis *in vivo*.

In addition to transgenic animal generation, we are also trying to transfect mammary epithelial cells directly *in vivo*. Mammary epithelial cells, specifically mammary Luminal Epithelial cells (LEC) are responsible for expression of milk protein at the time of lactation. A huge cell division event occurs starting from the early stage of pregnancy until advanced stage of pregnancy in the LEC. A possible successful delivery of transgene in this time period in the LEC will create integration and propagation of delivered transgene in this cell type which will eventually result in expression of transgene at the time of lactation. We are adopting various delivery methods and working with different transfection agents to successfully deliver transgene in the LEC. We have achieved limited success in transfecting LEC through virosome mediated transfection. We entrapped transgene, where expression of human interferon gama (hIFN-γ) is controlled by Buffalo β casein Promoter, in virosome created out of reorganized Sendai viral membranes known to contain Hemagglutinin-neuraminidase (HN) and fusion factor (F) on surface of virosomes which entail membrane fusion. We exploited such virosomes to breach epithelial cells of mammary gland for delivering transgene. Virosomes were delivered via milk duct of mice. Transgenes delivered in this way interact with cell and integrate in the genome at somatic level causing genomic alteration of somatic cells. This does not genetically alter the crucial germ line of the species, hence avoiding issues related to genetically modified organaisms (GMO) involving germ-line gene integration. We were successful in expressing human interferon gama (hIFN-γ) in the milk (~6.6ng/ml of milk) of mice.

For autologous germ cell (Gc) transplantation, one testis of the mice was electroporated with EGFP transgene and the contra lateral testis treated with busulfan. Electroporated testis was removed surgically (hemicastrated) after 5 days of electroporation and Spermatogonial stem cells (SSC) were isolated, and amplified *in vitro*. After ~ 20 days of busulfan treatment to remaining testis, the transgenic SSC amplified in culture were transplanted in the busulfan treated testis. Efficient colonization of transplanted SSC was seen. Sperm count, in epididymis of the transplanted testis was also observed to be increased as compared to that in epididymis of the non-transplanted. PCR result confirmed the EGFP transgene amplification from the DNA of epididymal sperm from transplanted testis but not from non-transplanted epididymal sperm. The transplanted mice also generated normal litter size. GCT-GFP F1 progeny confirmed the presence of transgene by PCR, Slot Blot and Western Blot. The testes of GCT-GFP F1 mice under UV light showed EGFP expression in donor derived spermatogenic colonies. This confirmed that direct testicular injection of busulfan creates a nice model of germ cell

depleted testis where germ cell transplantation can be achieved later.

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PUBLICATIONS, PATENTS AND TECHNOLOGY TRANSFER

A. ORIGINAL PEER-REVIEWED ARTICLES

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B. REVIEWS / PROCEEDINGS

1. Gupta SK (2016) Embryo implantation and pregnancy: Immunologic intricacies and strategies for its success. *Am J Reprod Immunol* **75**: 205-207.

2. Gupta SK, Malhotra SS, Malik A, Verma S Chaudhary P (2016) Cell signaling pathways involved during invasion and

syncytialization of trophoblast cells. *Am J Reprod Immunol* **75**: 361-371.

3. Gupta SK, Malik A, Arukha AP (2015) Ovarian and oocyte targets for development of female contraceptives. *Expert Opin Ther Targets* **19**:1433-1446.

4. Kaur H, Salunke DM (2015) Antibody promiscuity: Understanding the paradigm shift in antigen recognition. *IUBMB Life*. **67**:498-505

5. Khater S, Anand S, Mohanty D (2016) *In silico* methods for linking genes and secondary metabolites: The way forward. *Synthetic and Systems Biotechnol* **1**:80-88.

6. Kumar P, Bhaskar S (2016) Analysis of T Cell proliferating and polarizing potential of murine dendritic cells in allogeneic-mixed leukocyte reaction. *Bio-protocol* **6**: e1750.

7. Kumar P, Marathe S, Bhaskar S (2016) Isolation of genomic DNA from mycobacterium species. *Bio-protocol* **6**: e1751.

8. Shabareesh PRV, Kaur KJ (2015) Overview of the structural and functional impact of protein glycosylation in mediating protein protein interactions. *Trends in Carbohydrate Res* **7**: 30-40.

9. Singh A, Upadhyay V, Upadhyay AK, Singh SM, Panda AK (2015) Protein recovery from inclusion bodies of *Escherichia coli* using mild solubilization process. *Microb Cell Fact.* **14**:41.

10. Suri A, Jagadish N, Saini S, Gupta N (2015) Targeting Cancer testis antigens for biomarkers and immunotherapy in colorectal cancer: current status and challenges. *World J Gastroint Oncol.* **7**: 492-502.

11. Thakur R, Anand R, Tiwari S, Singh AP, Tiwary BN, Shankar J (2015) Cytokines induce effector T-helper cells during invasive aspergillosis; what we have learned about T-helper cells? *Front Microbiol* **6:** 429.

PATENTS APPLICATION / GRANTED AND TECHNOLOGY TRANSFER

1. Meena J and Panda AK (2015) A novel typhoid vaccine comprise of conjugate of Vi polysaccharide with flagellin
(Indian patent application 201611002743 filed on 25/01/2016)

2. Nandicoori VK, Soni V, Yogeeswari P and Sriram D (2015) Depletion of *M. tuberculosis* GlmU from Infected Murine Lungs Effects the Clearance of the Pathogen
(Indian patent application 3167/DEL/2015 filed on 01/10/2015)

3. Panda AK, Singh MS and Updhayay AK (2015) Process for obtaining bioactive recombinant protein from inclusion bodies
(Indian patent no. 267617 granted on 27/07/2015)

4. Sengupta S (2015) RECQL4, the DNA helicase mutated in rothmund-thomson syndrome, regulated mithochondrial function
(European patent no. EP 2398922 *granted on 29/04/2015; validated in UK)*

Technology Transferred

1. Process of high cell density fermentation (Technology transferred to Imgenex India Pvt. Ltd., Bhubaneswar, Odisha).

Rock Garden

AWARDS AND DISTINCTIONS

Dr. Chandrima Shaha was elected the Vice-president of the Indian National Science Academy.

Dr. Sagar Sengupta was elected as a Fellow of the National Academy of Science, India.

Dr. Satish K. Gupta was awarded Life Time Achievement Award-2016, from Indian Society for Study of Reproduction and Fertility, on 18 February 2016 at Ahmedabad. He received Dr. Subhash Mukherjee Oration an award from the Academy of Clinical Embryologists, on 20th September 2015, Kochi, Thiruvantapuram and was also awarded with the J.C. Bose Fellowship, from Department of Science and Technology, Government of India. 2015-2020.

Dr. Rajendra P Roy was elected as a Fellow of The Indian National Science Academy.

Dr. Nimesh Gupta was recommended for Ramanujan Fellowship of Govt. of India in the month of December 2015 (Not availed) and received Ramalingaswami Fellowship of Govt. of India (Availed) in January 2016.

Dr. Pushkar Sharma was elected as a Fellow of the Indian National Science Academy.

Dr.Lalit C Garg was elected as a Fellow of the Indian National Science Academy,

Dr. Akhil C Banerjea was selected for the Indian National Science Academy (INSA) - Senior Scientist Position - effective 1st January 2016.

Ph.D Degrees Awarded To NII Scholars

Twenty-five scholars of the Institute were awarded the degree of Doctor of Philosophy by Jawaharlal Nehru University on the completion of their work. The details are as follows:

Student's Name	Topic of Research	Guide
Mr. Akhade Ajay Suresh	Understanding regulation of TLR activation in immune cells	Dr. Ayub Qadri
Mr. Abhinav Shrestha	Evaluation of the Immunogenicity and Contraceptive Efficacy of the Gamete Specific Recombinant Proteins	Dr. Satish K. Gupta
Mr. Dipankar Ash	The role of mTOR complexes in regulation of autophagy in association with p53	Dr. Chandrima Shaha
Ms. Nuzhat Ahsan	Interventional studies on a-Synucleinopathies	Dr. Sarika Gupta
Mr. Hemant Jaiswal	Understanding the Role of Interferon Regulatory Factors in Dendritic Cell Differentiation	Dr. Prafullakumar B. Tailor
Mr. Yogesh Chawla	Delineating Serine/Threonine Protein Kinase B (PknB) Mediated Signaling in *Mycobacterium tuberculosis*	Dr. Vinay K. Nandicoori
Mr. Anupam Singh	Structural analysis of inclusion body aggregates	Dr. Amulya K. Panda
Ms. Richa Kapoor	Interplay between host microRNA34a and HIV-1 pathogenesis	Dr. Akhil C. Banerjea
Ms. Jasneet Kaur Khalsa	Studies on lineage-specific consequences of cellular Housekeeping functions	Dr. Satyajit Rath
Ms. Radhika Mathur	Understanding Sterol and Fatty Acid Involvement in Leishmania Survival	Dr. Chandrima Shaha
Mr. Praveen Kumar	Second Messenger Signaling Pathways in *Apicomplexans*	Dr. Pushkar Sharma
Ms. Shradha Khater	In silico identification of novel biosynthetic pathways: Applications to posttranslational modifications and biosynthesis of natural products	Dr. Debasisa Mohanty

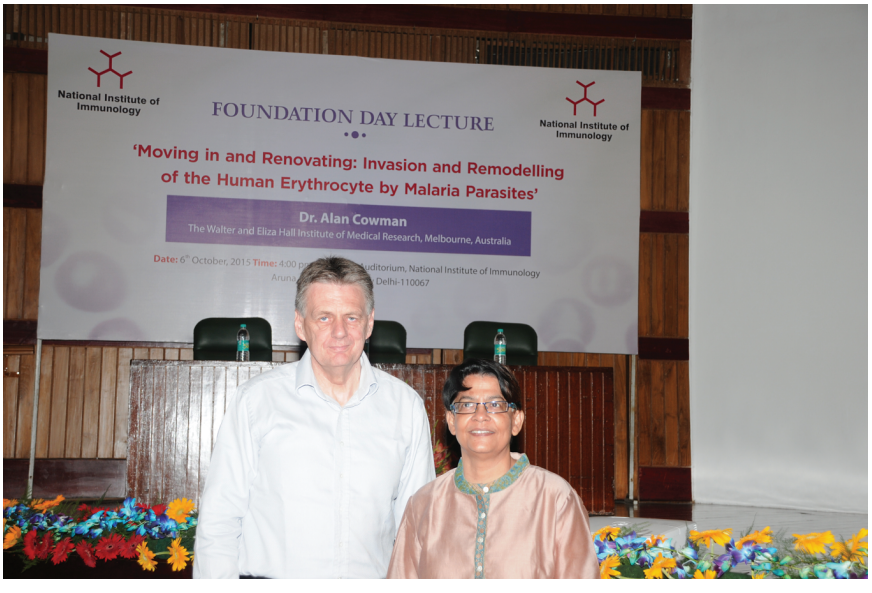
Student's Name	Topic of Research	Guide
Mr. Manish Chamoli	Molecular Genetic Characterization of a Novel Kinase that Regulates longevity in *Caenorhabditis Elegans*	Dr. Arnab Mukhopadhyay
Mr. Yatendra Kumar Satija	Exploring the molecular mechanisms underlying regulation and function of tumor suppressor p73	Dr. Sanjeev Das
Ms. Veena K	Role of allogeneic bone marrow progenitor cells in liver regeneration and study of genomic and epigenetic change involved in cellular reprogramming	Dr. Pramod K. Upadhyay
Ms. Priyanka Parijat	Stress Regulation and Persistence Mechanisms in *Mycobacteria*	Dr. Janendra K. Batra
Mr. PRV Shabareesh	Exploring the structural and functional impact of sugars in glycopeptidyl thrombin inhibitors	Dr. Kanwaljeet Kaur
Mr. Sanket Keshav Rane	Studies on cellular ageing in T cell lineage	Dr. Vineeta Bal
Ms. Shikha Singh	Sortase-mediated peptide ligation for chemo-selective engineering of multivalent proteins	Dr. Rajendra P. Roy
Ms. Jyoti Kumari	To determine the functions of RECQL4 in mitochondria	Dr. Sagar Sengupta
Ms. Jaya Bhushan	Functional characterization of Lipoproteins from *Streptococcus pneumoniae*	Dr. Devinder Sehgal
Ms. Abinaya Sundari- T	Molecular milieu orchestrating stem cells fate in skeletal muscle fibrosis	Dr. Sandeep Saxena
Mr. Iyer Srikanth Rajagopalan	Tissue Engineering of Recellularized Small-Diameter vascular Grafts	Dr. Pramod K. Upadhyay
Mr. Vaibhav Upadhyay	Effect of organic solvents on amyloid aggregation and solubilization and refolding of inclusion body proteins	Dr. Amulya K. Panda
Mr. Mohd. Syed Ahangar	Structural and biochemical studies of imidazoleglycerol phosphate dehydratase from *Mycobacterium tuberculosis*	Dr. Bichitra K. Biswal

Auditorium

LECTURES AND SEMINARS

FOUNDATION DAY LECTURE

On 6th October 2015, the 29th Foundation Day of NII was celebrated at the Institute. **Dr. Alan Cowman**, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia was invited as a Guest of Honour. He delivered a lecture on **"Moving in a Renovating: Invasion and Remodelling of the Human Erythrocyte by Malaria Parasites".**



Dr. Alan Cowman after the Foundation Day Lecture with Dr. Chandrima Shaha

SCIENCE DAY LECTURE

National Science Day was celebrated on 29th February 2016 at the Institute. **Prof. V. Nagaraja**, President, JNCASR, Bangalore delivered a lecture on **"Perturbation of topology modulation".**

Prof. V.Nagaraja delivering the National Science Day Lecture.

OTHER LECTURES

Dr. Gerald P. Schatten, Director, Pittsburgh development Center, Division of Development and Regenerative Medicine, University of Pittsburgh School of Medicine, Pittsburgh, USA delivered a lecture on **"Can Regenerative Medicine Involving Stem Cells Conquer Diseases and Aging?"** at the Institute on 14th January 2016.

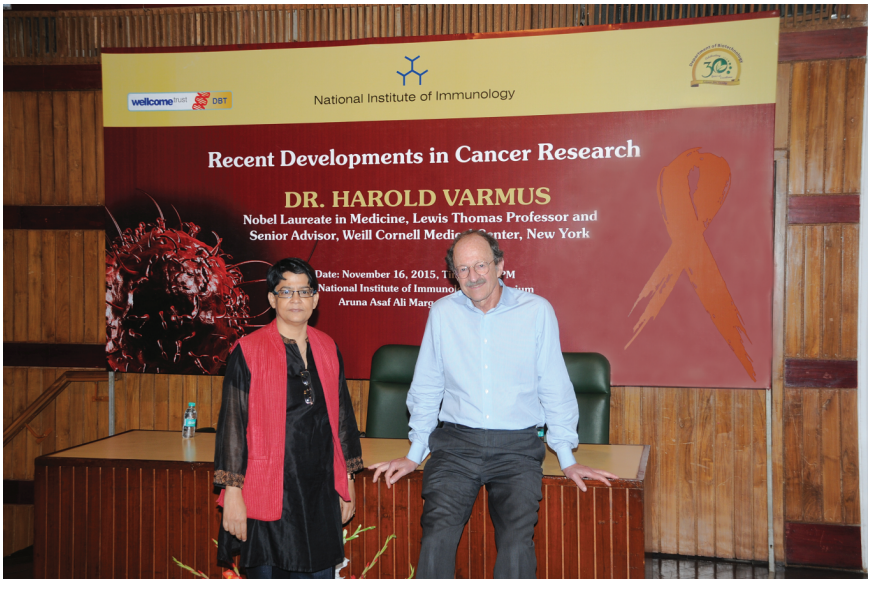
Dr. Gerald P. Schatten delivering lecture at NII auditorium.

Dr. Allan M. Weissman, Chief Laboratory of Protein Dynamics and Signaling, National Cancer Institute, NIH, USA delivered a lecture at the Institute on **"Ubiquitination at the Endoplasmic Reticulum: Insights into Metastasis and Ubiquitin Ligase Function"** on 25th January 2016.

Dr. Allan M. Weissman delivering lecture.

WELLCOME TRUST/DBT INDIA ALLIANCE PUBLIC LECTURES

Dr. Harold Varmus, Nobel Laureate in Medicine, Lewis Thomas Professor and Senior Advisor, Weill Cornell Medical Center, New York recognized for his research on retroviruses and the genetic basis of cancer delivered a public lecture on **"Recent Developments in Cancer Research"** at National Institute of Immunology (NII), New Delhi, on the 16th November 2015. The event was co-organized by NII, Wellcome Trust/DBT India Alliance and Department of Biotechnology, Government of India.



Dr. Harold Varmus with Dr. Chandrima Shaha

DNA@70 Public Lecture

Prof. Shankar Balasubramanian, Herchel Smith Professor of Medicinal Chemistry, Fellow of Trinity College, Department of Chemistry, University of Cambridge, co-inventor of a genome sequencing technology and co-founder of Solexa delivered a public lecture at the Institute on 17th June 2015. The lecture is an initiative of Wellcome Trust/DBT India Alliances' "DNA@70 Public Lecture Series". Prof Balasubramanian delivered a lecture on **' Decoding Human Genomes on a population Scale'**

Prof. Shankar Balasubramanian delivering lecture at NII auditorium.

SEMINARS

Sl. No.	Topic	Presented by	Date
1.	Mycobacterial lipid antigen presentation by CD1c and recognition by αβ and γδ T cells	**Dr. Sobhan Roy** Department of biochemistry and Molecular Biology, University of Chicago, Chicago, IL, USA	24th April, 2015
2.	Making Blood from Skin	**Dr.Kiran Batta** Stem Cell Biology Group Cancer Research UKManchester Institute, Manchester, UK	30th June, 2015
3.	The role of IL-15 and chronic inflammation in autoimmunity and metabolic syndrome	**Dr. Sheela Ramanathan,** University of Sherbrooke, Sherbrooke, Canada	25th June, 2015
4.	Physiological responses to biomedical device implantation: the role of neutrophils	**Dr. Siddharth Jhunjhunwala,** MIT, Cambridge, USA	15th July, 2015
5.	Cellular Innate Immune responses against virus infections	**Dr. Saumendra N. Sarkar** University of Pittsburgh Cancer Institute, USA	27th July, 2015
6.	Alternative splicing and its implication in cancers	**Dr. Samit Chattopadhyay** NCCS, Pune	28th July, 2015
7.	How genetic polymorphismscontribute to inter-individual variations in immune response ~ making SNPs make sense!	**Dr. Amit Lahiri** Yale University of Medicine, CT, USA	19th August, 2015
8.	Cancer "module"-omics: analyzing the network of cancer diseases and genes reveals "movers" and "shakers" of the disease	**Dr. Sitabhra Sinha** Institute of Mathematical Sciences, Chennai	27th August, 2015
9.	Influenza virus capsid disassembly: how a hard nut cracks itself to infect cells by carjacking molecular motors	**Dr. Indranil Banerjee** Novartis Presidential Postdoctoral Fellow Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland	30th September, 2015
10.	From synthesis to function - how nuclear history determines RNP architecture and dynamics in the cytoplasm	**Dr. Sanjay Ghosh** MRC Functional Genomics Unit University of Oxford, United Kingdom	08th October, 2015

Sl. No.	Topic	Presented by	Date
11.	Applications of Biomolecular Simulations- Identification of Protein Misfolding Mechanism and Prediction of Novel Nucleic Acid Analogs	**Dr. Atanu Das** Department of Chemistry University of Texas at Austin, USA	12th October, 2015
12.	Multiscale Modeling of Macromolecular Biosystems: Surmounting the Challenge of Bridging the Scales	**Dr. Parimal Kar** Michigan State University, USA	16th October, 2015
13.	Role of nitric oxide synthase in modulating immune response and its potential therapeutic implications	**Dr. Debashree Basudhar** National Cancer Institute, National Institutes of Health, Frederick, USA	26th October, 2015
14.	Virus-mediated Deregulation of Signal Transduction & Immune Surveillance: Implications in Host Pathogenesis & Cancers	**Dr. Shuvomoy Banerjee** Assistant Professor Amity Institute of Virology and Immunology, Amity University, Uttar Pradesh,	03rd November, 2015
15.	Modelling the role of nutrients in aging and age-related diseases using *D. melanogaster* and *C. elegans*	**Dr. Pankaj Kapahi** Professor, Buck Institute of Research on Aging, USA	18th November, 2015
16.	DNA Photodamage in the Dark: Mixed Blessings of Melanin (Double edged melanin biochemistry)	**Dr. Sanjay Premi** Dept. of Therapeutic Radiology Yale Cancer Center Yale University, New Haven, CT, USA	19th November, 2015
17.	NOSIP mediates immunomodulation by Nitric Oxide	**Dr. Balachandran Ravindran** Director, ILS, Bhubaneswar	23rd November, 2015
18.	Balancing tRNA synthetic rate and modification-dependent activity for condition-appropriate translation	**Dr. G. AneeshkumarArimbasseri** National Institutes of Health Bethesda,MD.USA	26th November, 2015
19.	The hypoxic tumor microenvironment: A driving force for breast cancer progression	**Dr. Pallavi Chaturvedi** Department of Medicine University of Illinois at Chicago, USA	30th November, 2015
20.	Epigenomic and Nuclear Receptor Signaling Integration in Cancer and Fibroids	**Dr. Debabrata Chakravarti** Professor, Northwestern University Feinberg School of Medicine, Chicago, USA	1st December, 2015

Sl. No.	Topic	Presented by	Date
21.	Some Ancient Mathematicians with a focus on ancient India	**Dr. Brishti Guha** Associate Professor, School of International Studies JNU, New Delhi	2nd December, 2015
22.	Regulating a chromatin remodeler: From modifications to submit architecture	**Dr. Arnob Dutta** Stowers Institute for Medical Research, Kansas City, MO, USA	9th December, 2015
23.	Advances in Comprehensive Proteome Analysis	**Dr. Robert L. Moritz** Head, Proteomics Research, Director, Seattle Proteome Center, Institute for Systems Biology, Seattle, WA, USA	15th December, 2015
24.	Irf8 directs stress induced autophagy in macrophages and promotes clearance of intracellular bacteria	**Dr.Monica Gupta** Wadsworth Center, NY State Department of Health, USA	18th December, 2015
25.	Development of Strategies for Protection against Tuberculosis	**Dr. Bappaditya Dey** Research Associate Tuberculosis Research Center School of Medicine, Johns Hopkins University	19th January, 2016
26.	Autophagy in Flavivirus Infection and Innate Immune Response	**Dr. Manjula Kalia** Research Scientist E Vaccine and Infectious Disease Research Centre, THSTI, India	20th January, 2016
27.	Understanding Cell Signaling: Model, Mechanism & Inference	**Dr. Sayak Mukherjee** Battelle Center for Mathematical Medicine Ohio State University, Columbus, OH, USA	27th January, 2016
28.	Toxin Antitoxin system in *Mycobacterium tuberculosis*	**Dr. Prabhakar Tiwari** Vaccine and Infectious Disease Research Centre, THSTI, Faridabad	3rd February, 2016
29.	Control of Systemic and Organ-specific Inflammation by Foxp3-associated Transcription Factors in Regulatory T cells	**Dr. Dipayan Rudra** AIM, Institute of Basic Sciences, Pohang, Gyeongsangbuk-do, Republic of Korea	4th February, 2016
30.	Hematopoietic Progenitors Maintenance in Drosophila	**Dr. Bama Charan Mondal** University of California, LA, USA	8th February, 2016
31.	Regulation of neutrophil recruitment to inflammatory sites by NLRP12	**Dr. Fayyaz Sutterwala** Associate Professor, University of lowa, Carver College of Medicine, USA	17th February, 2016

Sl. No.	Topic	Presented by	Date
32.	T cell immunity in influenza virus infection - basic perspectives of activation & regulation	**Dr. Avijit Dutta** Department of Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan	17th February, 2016
33.	Apoptotic regulation beyond the core pathway: Drosophila neural stem cells as a model of in vivo cell death	**Dr. Richa Arya** Massachusetts General Hospital Harvard Medical School, MA, USA	24th February, 2016
34.	Molecular basis of lipid antigen presentation by CD1c and its recognition by αβ and γδ T cells	**Dr. Sobhan Roy** University of Chicago, USA	3rd March, 2016
35.	Identification of genetic and non-genetic risk factors and understanding their cross-talk in the pathogenesis of inflammatory bowel disease	**Dr. Garima Juyal** SERB Young Scientist School of Biotechnology, Jawaharlal Nehru University, New Delhi	07th March, 2016
36.	Themis and PKC-eta in regulation of T cell receptor signaling and Treg activity	**Prof. Nicholas Gascoigne** National University of Singapore, Singapore	15th March, 2016
37.	"Curcumin" The Wonder Drug In Waiting	**Prof. G. Padmanaban** Former Director Indian Institute of Science, Bangalore	21th March, 2016
38.	Ontogeny of macrophages and dendritic cells	**Dr. Florent Ginhoux** SlgN, Singapore	23th March, 2016
39.	Is PfEMP1 universal to sequestration and severe malaria? Is looking beyond the key?	**Dr. Suchi Goel** Assistant Professor, Karolinska Institute, Sweden	30th March, 2016

CONFERENCE/SYMPOSIUM/ WORKSHOPS ORGANIZED AT NII

Digital India Week at NII

Digital India week was celebrated at the Institute form 1st to 7th July, 2015. A series of lectures were organized and Digital Repository of NII was also launched on 7th July, 2015. Mr. Prabir Mitra, NIC, DEIT delivered a lecture on Digital India and gave an excellent overview of the road map of Government of India for digital empowerment. Dr. Vinod Scaria, Senior Scientist, GN Ramachandran Knowledge Centre, CSIR-IGIB also gave a lecture. He highlighted the role of modern communication tools, digital technology and innovative methods like crowd sourcing in areas like genomics and BIG DATA analysis which require collaborative efforts from large number of researchers.

Mr. Prabir Mitra, NIC, DEIT delivered a lecture on Digital India

AlumNII@ Work: Interactions with NII Alumni

AlumNII@ Work: Interactions with NII Alumni was organized at the Institute on 5th October, 2015.

Winter NII Alumni Symposium

A Winter NII Alumni Symposium was organized at the Institute with a title " Molecular and Cellular body beyond the Rubicon: Graduating perspectives" on 23rd -24th December, 2015.

Science Setu

Workshop "Stem Cell Sciences and Application: Hype and Reality"

NII and five colleges of the Delhi University - Dayal Singh College, Gargi College, Institute of Home Economics, Sri Venkateswara College and Zakir Husain Delhi College - co-hosted a Workshop on Stem Cell Science and Technology: Hype and Reality on 22nd September, 2015 at the Salman Ghani Hashmi Auditorium in Zakir Husain Delhi College under the auspices of the NII Science Setu Programme. Dr. D. Balasubramanian, Director-Research, LV Prasad Eye Institute, Hyderabad delivered a lecture on 'Cells, Stem Cells and their Applications'.

Workshop on DNA Fingerprinting at Hindu College

Workshop on DNA Fingerprinting was organized at Hindu College under the auspices of National Institute of Immunology's Science *Setu* programme on 22nd January 2016. The

Workshop on DNA Fingerprinting at Hindu College

Workshop is being jointly organized by seven colleges on the North Campus of University of Delhi in collaboration with the National Institute of Immunology (NII). The workshop was organized in the background of the large-scale applications of DNA fingerprinting technologies in criminology, food &agriculture, medicine & health, veterinary science, conservation and classification of biodiversity. There was also a deliberate discussion on "The Human DNA Profiling Bill, 2015", proposed to be introduced to the Indian Parliament. Under the Bill, the government proposes to make DNA profiles of criminals and suspects across the country in a bid to bring down crime.

Dr. J. Gowrishankar, Former Director, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, graced the workshop as "Theme Speaker". There was a panel discussion on various aspects of DNA fingerprinting by experts and stakeholders.

DBT- EUROPEAN MOLECULAR BIOLOGY ORGANISATION (EMBO) JOINT PROGRAMME LAUNCH

The Government of India, through the Department of BioTechnology (DBT) has signed an agreement with the European Molecular Biology Conference (EMBC) and the European Molecular Biology Organisation (EMBO), inducting India as a member state of the EMBC and EMBO. The implications of this agreement for Indian science are far-reaching, it will provide impetus to the strengthening of links between Indian and European life sciences research. An official launch ceremony was held at National Institute of Immunology, New Delhi, India, on 4 February 2016. The kick-off event included scientific presentations by Nobel Laureates Christiane Nüsslein-Volhard and Ada E. Yonath.

Prof. K VijayRaghavan, Secretary, DBT and Prof. Maria Leptin, EMBO Director at DBT-EMBO Joint programme launch at NII auditorium.

As part of the agreement Indian scientists will be able to participate in the same EMBO programmes as researchers from all other member states:

EMBO Long-term/ Short-term fellowships
EMBO Courses and Workshops
EMBO Young Investigators Programme
EMBO Science Policy Programme
EMBO Global Meetings
The EMBO Meeting
EMBO Publications

The launch of this partnership was accompanied by a series of events across India. At each event scientists from Europe and India as well as EMBO representatives talked about science and opportunities afforded by the partnership.

INAUGURATION OF TRANSMISSION ELECTRON MICROSCOPY UNIT AT NII

The Institute has recently acquired and installed Electron Microscope. It is installed and fully functional at Transmission Electron Microsopy Unit with specialized technicians to operate it. Prof. K. VijayRaghavan, Secretary, DBT and Chairman, Governing Body, NII inaugurated this unit on August 18, 2015.

Prof. K. VijayRaghavan, Secretary, DBT and Chairman, Governing Body, NII, Dr. Chandrima Shaha and Senior Scientists during inauguration of Transmission Electron Microscopy on August 18, 2015.

NOTABLE ACTIVITIES

ACADEMIC COURSES, TRAINING PROGRAMMES AND INTERACTION WITH OTHER ACADEMIC INSTITUTES

The Institute imparts long term residential training leading to Ph.D. Degree of the Jawaharlal Nehru University, New Delhi. Every year 30-35 scholars are admitted to this Programme on competitive basis after an examination and interviews amongst a large number of applicants from all over the country.

The Ph.D. Programme of the Institute was launched in the academic year 1986-87. Since than the Institute has admitted a total of 641 students in 30 batches. So far 363 students have been awarded the Ph.D degree including 25 that have obtained the degree in academic year 2015-16. Many others are at various stages on their research work for the degree.

In addition, the Institute accepts students from various Universities/Institutions as Summer Research Fellowship Awardees under Science Setu and provides them facilities and guidance. Besides, the Institute also accepts students for the project work during the last semester of their Post Graduation course.

IMPLEMENTATION OF OFFICIAL LANGUAGE POLICY

The Official Language policy of the Govt. of India is followed by the Institute in letter and spirit:

To promote Hindi as Official Language in official work, Hindi Pakhwara (Hindi Fortnight) was celebrated in the Institute with great zeal from 1st to 14th Sept 2015. During this period, various Hindi competitions such as Hindi Sulekh (Hindi Writing), Hindi Nibandh (Hindi Essay), Hindi Shrutlek (Hindi Dictation), Hindi Vaad-Vivad (Hindi Debate), Hindi Samanya Gyaan (General Knowledge Competition) and Hindi Kavita Pathan (Hindi Poetry Recitation) were organized in the Institute, wherein a large numbers of faculty members, staff members and students had participated. Hindi Diwas (Hindi Day) was celebrated on 14th Sept, 2015 at the culmination of Hindi Pakhwara.

Hindi Workshop on **"Grammatical errors and its correction in Hindi Language** " was organized for the Staff members to remove the hesitation for carrying out their Official work in Hindi. As NII is a scientific and research Institution, therefore a lecture on "**Invisible pollution in Environment, its harmful effects and remedial measures"** was also organized on 12th January 2016 and Dr D.D. OJha, former

senior scientist of GWD Jodhpur and member of Official Language advisory Committee, Ministry of Science & Terminology, Department of Biotechnology & Ministry of Earth Science, New Delhi was delivered a lecture in Hindi.

Institute has implemented the Govt. of India incentive scheme for writing notes and drafts originally in Hindi by staff members. An incentive Scheme for encouraging and creating interest amongst Scientific and Technical staff members of NII for writing articles, research papers in Hindi on Scientific and Technical subjects was also implemented in the Institute.

Dr D.D. OJha, Shri N.S. Padmanabhan, Senior Manager, NII and Staff during Hindi Workshop

INDEPENDENCE DAY CELEBRATION

Independence Day was celebrated in the Institute on 15th August 2015. The event was marked by Independence Day Message from the Director, followed by singing of the National Anthem by the students and children of the staff of the Institute.

NII Staff celebrating Independence Day at the campus.

FAREWELL TO Ph.D STUDENTS

Farewell function of the 2010 batch of PhD Students with Staff Scientists, which was marked by planting of tree by the students at the Institute.

Group Photo of Ph.D 2010 batch

ANTI-TERRORISM DAY, SADHBHAVNA DIWAS AND COMMUNAL HARMONY WEEK

Anti-Terrorism Day was observed by all employees of the Institute on 21st May 2015 by taking anti-terrorism/violence pledge stating: 'We, the people of India, having abiding faith in our country's tradition of non-violence and tolerance, hereby solemnly affirm to oppose with our strength, all forms of terrorism and violence. We pledge to uphold and promote peace, social harmony and understanding among all fellow human beings and fight the forces of disruption threatening human lives and values'.

With the theme to promote national integration and communal harmony among people of all religions, languages and regions, 'Sadhbhavna Diwas' was observed in the Institute on the birth anniversary of late Shri Rajiv Gandhi on 20 Aug 2015 by taking pledge by each staff that 'I take this solemn pledge that I will work for the emotional oneness and harmony of all the people of India regardless of caste, region, religion of language. I further pledge that I shall resolve all differences among us through dialogue and constitutional means without resorting to violence'. To promote the idea further a fortnight from 20 August to 3 September 2015 was observed as Communal Harmony Week.

REPRESENTATIONS OF SCHEDULED CASTES, SCHEDULED TRIBES, OTHER BACKWARD CLASSES

The Institute follows reservation orders as per directives of Government of India, while making appointments, to ensure representation of Scheduled Castes, Scheduled Tribes and Other Backward Classes as per the prescribed percentage. During the reporting period, 3 vacancies reserved for Scheduled Castes and 2 vacancies reserved for Other Backward Classes (OBC) were filled.

REPRESENTATION OF PERSONS WITH DISABILITIES

The Institute follows reservation orders for Persons with Disabilities as per Government of India directives issued from time to time to ensure representation of persons with disabilities as per the prescribed percentage. During the reporting period, one Group “A” backlog vacancy was filled by a Scientist, who has since joined the Institute. Action has also been initiated to fill-up one Group 'C' vacancy reserved for PWD-Hearing Impairment. Selection Committee has been constituted and the outcome would be informed in next Annual Report.


EKTA MARKETING CORPORATION
DECEMBER
PROTECTO

INFRASTRUCTURE

RESEARCH FACILITIES

EQUIPMENT

While most of the routine equipment is available in various laboratories of NII, some high-end instrument facilities are shared by various research groups and their collaborators. The equipment in these facilities includes Mass Spectrometers, NMR Spectrometers, Confocal Microscopes, Atomic Force Microscope, Scanning and Transmission Electron Microscopes, High Throughput DNA Sequencer, Flow Cytometers, Dual wavelength X-ray Generator and X-ray device for in-vivo imaging.

BSL-III FACILITY

Tere are three Biosafety Level III facilities at NII- one each for handling Mycobacterium tuberculosis, Streptococcus pneumonia and HIV.

SMALL ANIMAL FACILITY

The Small Animal Facility of the Institute is committed to ensure the humane care of animals used in approved research and cater defined strains of mice and rats to the scientific community of the institute. At present the facility holds 93 mouse strains, 6 rat strains and 1 stock of rabbit.

The propagation of all defined strains is done in a three- tier system i.e., the Foundation Stock (FS), Pedigreed Expansion Stock (PES) and Production Stock (PS). Genetically modified mouse strains are bred either by 1.Homozygous mutant (-/-) x homozygous mutant (-/-) 2. Heterozygous mutant (-/+) x homozygous mutant (-/-) 3.Heterozygous mutant (-/+) x heterozygous mutant (-/+)

Defined breeding protocols and careful management and husbandry procedures are followed to ensure the purity of each strain of mice. To maximize genetic purity and uniformity of mice, inbred strains are propagated and replaced periodically in such a manner that minimizes the genetic drift and inbreeding depression. A random sample from few breeders of Foundation, Expansion and Production stock are monitored with the help of few microsatellite and biochemical markers to ensure their genetic purity. The facility also gets support from various principal investigators in the genotyping of transgenic and knockout mice strains to confirm the genetic purity based on presence or absence of the selected gene of interest.

Health monitoring program includes regular screening of pathogens that includes Mouse Hepatitis virus, Mouse Parvovirus, Mouse Norovirus ,Pnemonia virus of Mice,

Mycoplasma and Sendai virus using Elisa and PCR. Bacterial pathogens such as *Pseudomonas aeroginosa, Streptobacillus moniliformis, Bordetella, Bronchiseptica, Citrobacter rodentitium, Pasteurella pneumotropica, Staphylococci* and *E.Coli* are screened using culture, biochemical and PCR methods. Faecal samples are randomly selected for the presence of endoparasites by sedimentation method for the presence of syphacia and aspicularis species. Also periodic FACS analyses are also done on immunodeficient mice for their leakiness.

The health quality procedures are implemented to prevent the transmission of infection between cages, which include careful handling of animals, washing using automated cage and bottle washer, use of sterilized corn cob bedding, autoclaved cages, and acidified autoclaved drinking water. The breeding colonies are maintained in IVC systems of international standards. Necessary action based on clinical signs is taken by the veterinarian concerning the necropsy/ autopsy of the infected animals. Preventive and recommended schedule of medication is strictly followed to prevent the infection/s.

PRIMATE RESEARCH CENTRE

The National Institute of Immunology has a separate facility of Primate Research Centre. Macaques are bred and maintained in the Primate Research Centre for generation of in-house animals of known ages for approved basic, pre-clinical and toxicological research using sub-human primates.

Group mating is done, under the breeding program, for the production of healthy animals. This helps in providing animals of known age and parentage. We have large open pens, which are used for group mating under semi-natural conditions where food and water is provided ad libitum. Infants are weaned at the age of six months after which they are transferred to open semi-natural housing for over-all growth and better development of bones, muscles and coordination. Monkeys are housed in independent cages at around pubertal age. To prevent cross-cage contamination strict procedures are followed. All cages are washed routinely by scrubbing with soap and are painted once a year. Deworming of the colony is done at least once a year. To check outbreak, the routine TB tests are performed because non-human primates are susceptible to this infection. The chest x-ray of animals, doubtful of the infection, is performed using x-ray machine and dark room of the Centre. The sick animals are isolated and treated properly after pathological investigations and veterinary consultation according to international norms. To treat the minor injuries, gastrointestinal disorders and to revive animals during acute cardio-pulmonary crisis, a stock of medicine is maintained at the Centre.

Protein rich pellets containing appropriate content of fat, carbohydrate and vitamin are provided to monkeys ad libitum. In addition to this, bread, germinated gram, vegetables and/or fruits are also given daily. For change of taste, occasional feast like bread with sauce or jaggery coated groundnuts are given. Breast feeding mothers and pregnant females are given calcium and vitamin supplements on bread. Care is taken to provide excess feed to such females. Drinking water is provided to the animals by pipelines behind monkey cages, which are connected to flexible protective hose-pipe at the top of each cage. Steel nozzles with Teflon interior are fitted at the tip of these hosepipes for the continuous access to drinking water. To make the staff aware of or to remind preventive measures for health safety, occasional meetings are held with the staff and they are mentored very often. The attendants are provided with overall, jacket, pyjama and footwears for use during animal handling and cleaning. Use of gloves and mask is mandatory

during work. Booster of TT is given once every year. The staff also receives boosters of anti rabies vaccine when required. TB test and chest x-ray of staff and the security personnel are performed occasionally. As a preventive measure, persons having injury are given non-animal work. Every precaution is taken to prevent cross species infection; monkey to human and vice versa. High-grade sanitary norms are followed for cleaning in the monkey rooms and area surrounding the building by using disinfectants and insecticides. To prevent colonization of microbes the sewer channels and tiles of room are routinely cleaned.

Major surgeries are performed in the well-equipped operation theatre whereas minor surgeries involving cuts and wounds are performed in the animal prep room adjacent to it. Technical expertise for surgery, immunization, bleeding, biopsy, electro ejaculation and fertility studies is extended in addition to maintaining and providing primates free of microbial pathogens. Surgical linen is washed using a washing machine. Autoclaving facility for surgical equipments and accessories is provided within the building. A research laboratory is situated in the centre for the research related to primates and the samples obtained from them. This provides basic services to various investigators involving primary processing of biological samples in the Centre.

At this centre, clearance of the research proposals by CPCSEA after primary clearance from the Institutional Animal Ethics Committee, comprising of scientists from various fields of expertise and member of CPCSEA is a necessary requirement for conducting research on primates. The macaques at this Center served research related to infectious diseases, reproduction, endocrinology, immunology and contraception. The staffs of Center makes sure that all the procedures involved in animal handling are pain-free and involve minimum stress to the animal. Where ever unavoidable, proper medication is given to reduce the pain. Experimental animals are provided with special feed, whenever needed. A constant effort is made to keep the animals in comfortable and stress free environment as per the available guidelines. There are seventeen open enclosures with swings and shelters, some of these are used for rotation of monkeys and some for rehabilitation and or socializing. Attempts are made to keep monkeys in groups in the open enclosures.

SUPPORTING UNITS

SUPPORTING UNITS

Establishment, Personnel and General Administration Services

The Division continued to provide key support through relentless efforts for optimally utilizing and intergrating human and administrative resources aimed at realizing the vision of the Institute. During the reporting period effective administrative support was provided for formulating policies and ensuring their effective implementation. Other key areas include handling service matters, recruitments, career development, foreign visit of scientists for training/conferences/bilateral exchange visits ets. staff welfare, post retirement dispensation, preparation and submission of periodic reports to the administrative ministry, liaisoning with them and handling Parliament Questions. To bloster the capabilities and enhance productivity, the Institue conducts periodical training for its Adminstrative and Technical Staff by way of in-house training imparted by experienced professionals as well as by sponsoring them for training in recognized training institutes.

During the reporting period, the Institute initiated efforts for digitizing employees records for implementing e-governance and has been successful in porting employees' data in electronic form using latest state of the art ICT solutions. The e-governacne portal, INTRANII, would facilitate employees to view their personal and financial details.

Financial and Accounting Services

The division has been responsible for preparation of annual budget, management of funds utilization, receipt and disbursement of all payments, internal auditing, getting accounts audited by statutory and CAG auditors, sending reports to funding agencies and recovery and remittance of TDS from salary and contractors, filling institutional income tax return, obtaining required exemptions of the Income Tax department, maintaining bank accounts, management of trust for CPF, Gratuity Fund, and recovery and remittance of subscriptions of NPS.

Stores and Purchase Department

The Stores & Purchase Department of the Institute is responsible for all purchases such as chemicals, consumables, research equipment and instruments, glassware and other items. It acts as lifeline for research activities. Special emphasis is laid on economic and timely procurement of stores and supplies from local as well as international sources. The important function of purchase is overseen by various purchase committees comprising of three or more scientists, Finance & Accounts Officer and Stores & Purchase Officer. The officials of the Stores Department carry out the processing of

orders and procurement of materials of different types for the Institute and distribute them to the concerned labs on receipt.

Engineering, Maintenance and Instrumentation Services

The Engineering department of the Institute has been entrusted with all the engineering activities involving maintenance, services and capital works. It has always been the endeavour of the department to provide the best of services with use of the latest/modern technology; as a result systems are being continuously modernized. Major activities under taken during the reporting year are as follows:

(i) Construction of boundary wall at Sector V Dwarka. (ii) Miscellaneous works in PRC at NII (iii) Setting up of New Laboratories & Offices (iv) Creation of warm culture Room. (v) Servicing/repairing of various DG Sets to maintain back up supply in healthy condition. (vi) Miscellaneous civil works in vaccine Immunology & Metabolic Research Labs at NII (vii) providing & fixing of frameless toughened glass at enterance of Auditorium & Cafeteria at NII (viii) Redesigning the space of erstwhile administration at NII (ix) Miscellaneous works in newly allotted lab space at NII. (x) Ducting & associated work in vaccine Immunology & Metabolic research lab at NII (xi) Replacement of defective cooling coil of AHU in different laboratories at NII.

The department is currently working on the following projects:

(i) Installation of rain harvesting system at NII (ii) Supply, Installation, Testing & commissioning of 1750 KVA D.G. Set at NII (iii) Installation of roof top grid sharing solar system at NII. (iv) Setting up of New Laboratories & Offices at NII. (v) Installation of LED lighting fixtures & retrofit LED lamps in existing fixtures at NII. (vi) Installation of CCTV cameras at NII (vii) Redesigning of security Hut space at NII. (viii) Replacement of PVC fills of cooling towers at NII. (ix) Miscellaneous HVAC's work in small animal facility at NII.

Library and Documentation Services

Library And Documentation Department is a service oriented supportive Unit works as a Knowledge Management Centre. It provides information support to the scientific staff of the Institute using both archival and contemporary digital resources.

Institutional Repository maintaining in Dspace for archiving peer reviewed journals articles produced by NII researchers. This can be viewed at : http://www.nii.res.in/research/library.

Apart from that, Library has computerized all its housekeeping activities and are being maintained and updated regularly. Web-Online Public Access Catalogue (Web-OPAC) is available for searching database.

Library has a rich collection of books and journals. Library has made available Electronic resources on the desktop of the scientists. The E-journals are available under NII subscriptions and DeLCON consortium project to NII members on intranet/LAN. Library involved in the process of compilation of Annual Report of the Institute and purchasing process of print and online Journals, books and publications.

Library takes care of all binding and photocopying work of Institute. A Hindi Library with good collection of administrative Hindi Books and magazines has been set up for popularizing the official language amongst staff of the Institute.

Academic and Training Services

The activities of the Academic & Training Department have three major groups viz. Students Affairs, Outside Training and In-House

training. The Academic Department has been involved in Ph.D Admissions, Pre-PhD registration courses, Doctoral Committee meetings, Academic Committee meetings, Fellowship of scholars etc. The Institute intake the scientist who get their own fellowship from the from the following Institute/organisation under the respective guides of the Institute. Indian Institute of Science Bangalore (DBT-RA),ICMR (SRF/RA), Department of Science and Technology (DST-SERB, DST-Inspire Faculty, DST (WOS) and CSIR (SRA/RA). The Institute also impart short-term training to the fellows sponsored by the India Academy of Science Bangalore, students coming from Toronto University Canada under Indo-Canadian collaboration and under-graduate students coming from different colleges under Science Setu programme. The Department has also been involved in arranging the participation of Scientific, technical and administrative officials of the Institute in the training courses, workshop and seminars organized by outside organization in different parts of the country.

Vigilance Cell

The Institute has a Vigilance Cell headed by a Scientist nominated as part-time Chief Vigilance Officer (CVO) by the Central Vigilance Commission (CVC). The CVO and the support staff perform vigilance functions as adjunct duties in addition to their primary responsibilities. The Cell has effectively followed various instructions issued by the CVC from time to time to ensure effective implementation of the measures outlined in the instructions for strengthening vigilance and anti-corruption work. Emphasis has been laid primarily on preventive vigilance since such vigilance, if properly conceived and executed aids in plugging weak and vulnerable areas. The Institute has been reviewing existing procedures to identify corruption prone areas, making policies more transparent to avoid ambiguity and streamlining procedures to achieve a corruption free environment. Plans for rotation of staff employed in sensitive areas prone to corruption have been implemented from time to time. Sizeable purchases of chemicals, consumables and instruments are handled through various purchase committees of the Institute, thus eliminating the possibility of collusion detrimental to quality and price of purchases. Periodically, the composition of the purchase committees is reviewed and the committees are reconstituted. The Cell has been rendering periodical reports and returns on vigilance activities to the administrative machinery and CVC.

'Vigilance Awareness Week' was observed in the Institute from 26th to 31st October 2015 during which a pledge to fight against corruption was taken on 26th October 2015. Shri R. N. Nayak, Officer-on-Special Duty from Central Vigilance Commission gave a talk on 'Preventive vigilance as a tool for good governance' as a part of the Vigilance Awareness Week.

Computer Centre

Computer Centre has been providing all Information Technology related support to NII, which involve managing switches and Wi-Fi controllers in a 650 node LAN, system administration of multiple LINUX based E-mail and Web servers, backup services for mail/web servers, managing UTM devices for network security and integrating internet bandwidth from multiple ISPs. Computer center staff facilitates day to day trouble shooting, maintenance and anti-virus support of about 800 PCs and other peripheral devices. In addition Computer center also provides specialized services like management of HPC clusters, managing floating licenses for access to Bioinformatics softwares over LAN and IT support for developing in house software for Pay Roll and maintenance of employee database.

ORGANIZATION

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Senior Science &
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(Nominee of Vice Chancellor, JNU)

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(as on 31.03.2016)

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Deputy Director
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(retired on 30/04/2015)
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(retired on 30/04/2016)
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Dr. Vivek Tripathi
Dr. Anurag Misra
Dr. Upasana Bedi
Dr. Chaitali Banerjee

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Dr. Sakshi Gupta
Dr. Md. Mashkoor Alam

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Ms. Abinaya Sundari T.

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DST- Inspire Faculty Award
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DST SERB (Post Doctoral Fellow)
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Mr. Ramesh Kumar
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Technician II
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Mr. Rajinder K. Thapa
Mr Rajesh Kumar

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Mr. Veer Bhan

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Mr. Yash Pal Singh
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Mr. Mohar Singh
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Mr. Prem Chand
Mr. Ram Bhool
Mr. Ram Dev Yadav
Mr. Ram Surat
Mr. Subhash Chand III

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Ms. Chandresh Bhagtani
Ms. Lalitha Nair

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Mr. Ranbir Singh

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Ms. Sheela Satija
Ms. Daisy Sapra

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Mr. Dev Datt Sharma
Mr. Jagdish Mogha
Mr. Sant Lal
Mr. Siddharth Sharma

Junior Hindi Translator
Ms. Nisha

Junior Assistant II
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Mr. Madan Lal
Mr. Mahender Singh
Mr. Satyabir Singh
Mr Suti Prakash

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Mr. Rajeev Kumar
Mr. Ajay Kumar

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Mr. Pradeep Chawla

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Mr. Aslam Ali

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Mr. Pradip K. Sarkar

Technician II
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Section Officer
Mr. Mahender Pal Singh

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Mr. Dharambir
Mr. Than Singh
Mr. Om Prakash

Junior Assistant I
Mr. Alam Singh

Junior Assitant II
Mr. Daya Chand

Skilled Work Assistant
Mr. Sandeep Singh Kuswaha

Photographs/images on the separators are contributed by
Dr. Agam P. Singh, Dr. Vinay K. Nandicoori and Dr. Bichatra K. Biswal.

A page from history

Prof. Yash Pal inaugurating the Primate Research Facility of NII on March 14th 1986 with Prof. G.P. Talwar, Founder Director.

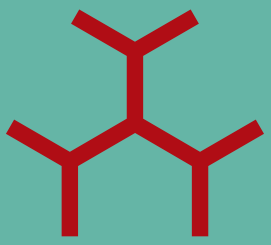
NII Collaborations

1	Aligarh-	JN Medical College, AMU
2	Allahabad-	University of Allahabad
3	Bangalore-	IISc, IOB, Christian Blind Mission, IBAB, St John's Medical College, NIMHANS, St. Johns Research Institute, JNCASR
4	Bhubaneswar-	CIFA ,KIT, ILS,
5	Chandigarh-	IMTECH
6	Chennai-	Cancer Institute (WIA)
7	Delhi/NCR-	AIIMS, University of Delhi, JNU, THSTI, ILBS, St. Stephen's Hospital, Jamia Hamdard, CSIR-IGIB, TRF, I CARE Eye Hospital, SH & VMMC, RCB, VIMHANS Hospital, NIP, RML Hospital, IOP, UCMS/GTB Hospital, IIT Delhi, ICGEB INMAS, FARMER
8	Hyderabad-	BITS, University of Hyderabad, CCMB, CDFD
9	Jaipur-	MG Medical College and Hospital, University of Rajasthan
10	Kanpur-	IIT-K,
11	Kalyani -	NIBMG
12	Kolkata-	IICB
13	Lucknow-	CDRI, SGPGI
14	Mohali-	IISER M
15	Mumbai-	NIRRH, ACTREC, TIFR
16	Pune-	CSIR-NCL, NCCS, IISER, BAIF, TCS
17	Sagar-	DHG Vishwavidyalaya
18	Shantiniketan-	Visva-Bharati
19	Tezpur -	Tezpur University
20	Thiruvantapuram-	HLL Lifecare Ltd
21	Vadodara-	MSU of Baroda
22	Varanasi-	BHU Medical College

NII Collaborations

1	Southwestern Medical Center, Dallas, USA
2	University of Arkansas, Fayetteville, USA
3	St. Louis University, St. Louis, MO, USA
4	National Institute of Health, Bethesda, MD, USA
5	Harvard School of Public Health, MA, USA
6	North-Eastern University, Boston, USA
7	Seattle Proteome Centre ,USA
8	Alnylam Pharmaceuticals, Massachusetts, USA

9	University of Buffalo, The State University of New York, Buffalo, USA
10	Imperial College, London
11a	LBCMCP-CNRS, Toulouse,
11b	Institute of Pasteur, Paris, France
12	University of Geneva, Geneva
13	Bernhard-Nocht-Institut für Tropenmedizin, Hamburg, Germany
14	University of Pretoria, Pretoria, South Africa
15	Shanghai Institute of Planned Parenthood Research, P. R. China
16	Monash University, Melbourne, Australia



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