National Institute of Immunology

Annual Report
2016-17

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CONTENTS

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's been an exciting year at the National Institute of Immunology, and it has been a pleasure and a privilege to observe proceedings from a unique vantage point.

Since its inception, NII has followed a carefully-considered intellectual path, one that has fostered the emergence of a very special research environment. While we enthusiastically explore basic biological processes and function, we are also constantly assessing the potential of novel medical intervention; frequently, these pursuits represent a continuous and overlapping spectrum. The few chosen examples below are enumerative:

While we strive to understand the mechanisms contributing to the uncontrolled growth of cancer cells, we also investigate ways to prime the immune system in order that such growth can be curtailed in a specific and targeted way. While we seek to understand the factors responsible for microbial virulence and elucidate signalling pathways that enable the persistence of infection, our interests also lie in the development of vaccines aimed at preventing disease, or at treating individuals in whom infection has taken hold. Vaccine failure the world over is frequently linked to component instability; studies carried out over the last year, employing novel processes that enhance the stability of human-approved adjuvants can have a significant impact on human health. While correlation of structure with function (of both self and pathogen-derived proteins) serves to satisfy an intellectual urge, it can also aid in the design of new drugs, as emerging investigations demonstrate. Work on the factors that influence longevity and aging at both the cellular and organismal levels have helped in the understanding of natural processes that compromise physiological well-being at so many levels. The ability to 'trans-differentiate' cells traditionally considered of fixed phenotype and function to cell-types of an alternative, desired lineage, while a fascinating endeavour in its own right, has significant implications for transplantation medicine. And finally, work aimed at furthering our understanding of the (intrinsic and extrinsic) factors that influence the make-up and functioning of different components of the immune system continue to elucidate, and suggest corrective measures for, aberrant immune states.

I would like to acknowledge the elements that have contributed to our growth - stellar core and project scientific staff, a vibrant and energetic student community, constantly-upgraded infrastructure (including a state-of-the-art experimental animal facility), all backed up by extremely efficient administrative and technical services.

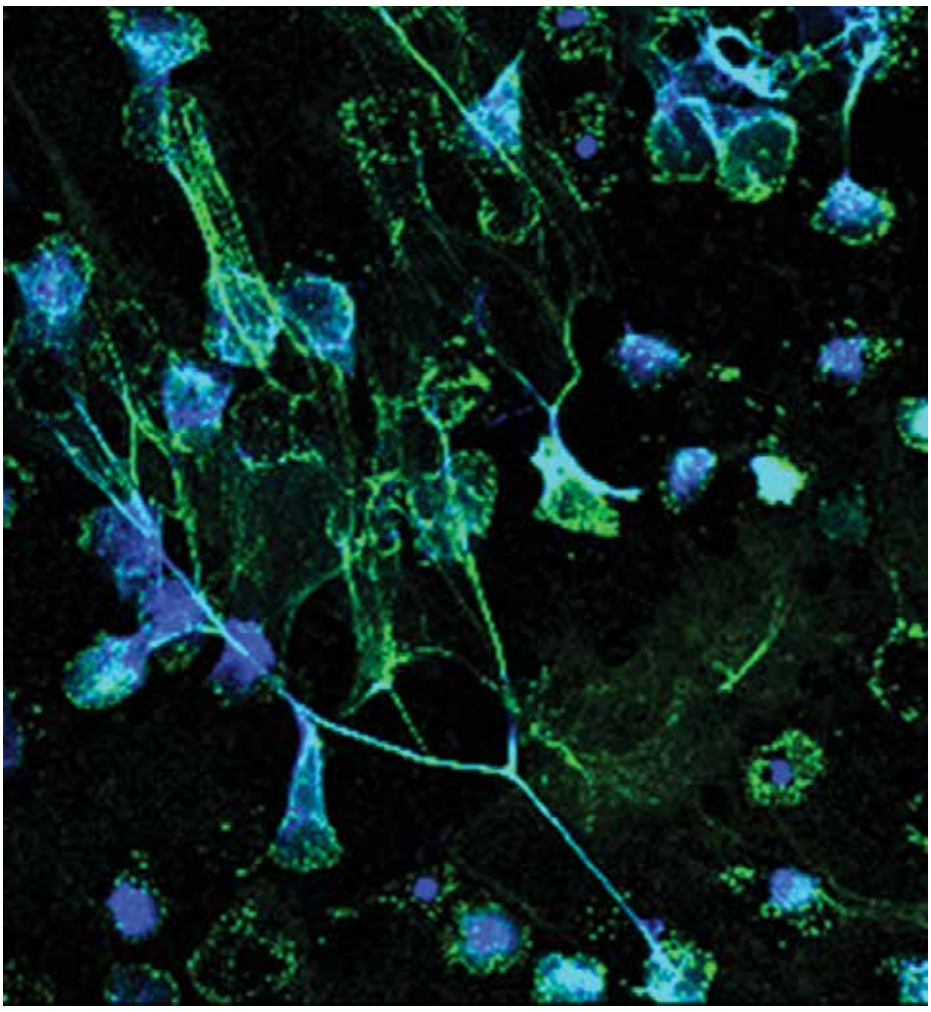
As is necessary in any successful endeavour, we frequently cast a critical eye on our efforts. Additionally, we welcome the critical feedback we receive during annual meetings the RAPSAC, and are grateful for the guidance and encouragement these interactions provide. Meetings of the Governing Body are further occasions for vital institutional appraisal. Generous core and extramural

funds, provided by the Department of Biotechnology as well as other government agencies, have been instrumental in our development and evolution.

While conscious of our institutional mandate, it is our intention to continue to carry out cutting-edge biological research of societal relevance, with approaches that will be increasingly theme-based. An over-arching goal of these pursuits will be the continued investigations into the workings of the immune system to further understanding of its functioning in health and disease. We believe the development of new vaccines against communicable and non-communicable diseases will be a natural corollary of these efforts, which will also make possible a finer dissection of autoimmune pathology. Indeed, we seek to nurture an environment conducive to the growth of both basic and applied scientific research in modern biology, fostered by ever-expanding links with the clinic and with industry. In appreciation of the fact that collaborative enterprise is increasingly becoming an essential component of modern science, we will reach out with renewed vigour to laboratories and universities across the world in the pursuit of shared research interests; such programmes will complement our skills and enhance our capabilities, steering us to that cherished ideal - a laboratory without borders. Besides providing a stimulus to intellectual enterprise, it is our fervent hope that such synergies will also shorten the bench-to-bedside interval for promising interventions that can contribute to the betterment of human health.

Dr. Anil Kumar Suri
Director (Additional Charge)

RESEARCH REPORTS



Neutrophil extracellular traps released from activated human neutrophils were visualised by probing for myeloperoxidase (Alexa Fluor 488, green). DNA was stained with DAPI (blue).

IMMUNITY AND INFECTION

Analysis of antigen processing and presentation

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The aim of the ongoing programmes in this group is to examine the generation and activation of T, B, and antigen-presenting myeloid cells using multiple interlinked experimental systems.

A variety of experimental approaches are taken to address the theme issues. The approaches currently used examine APCs and pathways involved in antigen presentation to MHC class I and class II-restricted T cells, and analyse the consequences of intracellular signal transduction modulation for both development and responses of B cells, T cells, and macrophages using genetic as well as pharmacological tools, in both mouse and human immune systems.

Cell-intrinsic genetic regulation of memory T cell pool size

Memory T and B lymphocyte numbers are thought to be regulated by recent and cumulative microbial exposures. We report here that memory-phenotype lymphocyte frequencies in B, CD4 and CD8 T-cells in 3-monthly serial bleeds from healthy young adult humans were relatively stable over a one year period, while recently activated B, CD4 and CD8 T-cell frequencies were not, suggesting that recent environmental exposures affected steady state levels of recently activated but not of memory lymphocyte subsets. Frequencies of memory B and CD4 T cells were not correlated, suggesting that variation in them was unlikely to be determined by cumulative antigenic exposures. Immunophenotyping of adult siblings showed high concordance in memory, but not of recently activated lymphocyte subsets, suggesting genetic regulation of memory lymphocyte frequencies. To explore

this possibility further, we screened effector memory (EM)-phenotype T cell frequencies in common independent inbred mice strains. Using two pairs from these strains that differed predominantly in either CD4EM and/or CD8EM frequencies, we constructed bi-parental bone marrow chimeras in F1 recipient mice, and found that memory T cell frequencies in recipient mice were determined by donor genotypes. Together, these data suggest cell-autonomous determination of memory T niche size, and suggest mechanisms maintaining immune variability.

We now intend to undertake a systematic examination of the genetic factors regulating memory T cell pool sizes, as well as the pathophysiological consequences of such quantitative variation in memory T cell pool sizes, especially to examine if increased occupancy of niche space by memory cells restricts the naïve T cell repertoire and confers any disadvantage in primary infections. We will also set up collaborations to begin examining the inter-individual variation in the rate of accumulation of memory from infancy to adulthood in outbred mammalian populations, and to explore if heterogeneity in quantitative variation of memory T and B cells might confer survival advantages for the population.

Publications

Original peer-reviewed articles

1. Jain N, Khullar B, Oswal N, Banoth B, Joshi P, Ravindran B, Panda S, Basak S, George A, Rath S, Bal V, Sopory S (2016) TLR-mediated albuminuria needs TNFα-mediated co-operativity between TLRs present in hematopoietic tissues and CD80 present on non-hematopoietic tissues. *Dis Model Mech* **9**: 707- 717.
2. Basu S, Kaw S, D'Souza L, Vaidya T, Bal V, Rath S, George A (2016) Constitutive CD40-signaling calibrates differentiation outcomes in responding B cells via multiple molecular pathways. *J Immunol* **197**: 761- 770.
3. Prabhu SB, Rathore DK, Nair D, Chaudhary A, Raza S, Kanodia P, Sopory S, George A, Rath S, Bal V, Tripathi R, Ramji S, Batra A, Aggarwal KC, Chellani HK, Arya S, Agarwal N, Mehta U, Natchu UC, Wadhwa N, Bhatnagar S (2016) Comparison of human neonatal and adult blood leukocyte subset composition phenotypes. *PLoS One* **11**: e0162242.
4. Balyan R, Gund R, Ebenezer C, Khalsa JK, Verghese DA, Krishnamurthy T, George A, Bal V, Rath S, Chaudhry A (2017) Modulation of naïve CD8 T cell response features by ligand density, affinity and continued signaling via internalized TCRs. *J Immunol* **198**: 1823-1837.
5. Jain N, Oswal N, Chawla AS, Agrawal T, Biswas N, Vrati S, Rath S, George A, Bal V, Medigeshi GR (2017) CD8 T cells protect adult naive mice from JEV-induced morbidity via lytic function. *PLoS Negl Trop Dis* **11**: e0005329.

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Understanding the role of Interferon regulatory factors in dendritic cell development and innate immunity

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Dendritic cells (DCs) are composed of multiple subsets that collectively provide early innate immunity leading to subsequent adaptive immunity. Plasmacytoid dendritic cells (pDC), $CD4^+$DC, $CD8\alpha^+$DC and $CD4^-CD8^-$DC are four major subtypes in the murine spleen. These subtypes of DCs express different sets of genes and assume distinct functions. Members of Interferon regulatory factors (IRFs) play critical role in DC development and functions. Interferon regulatory factor 4 (*Irf4)* and Interferon regulatory factor 8 (*Irf8)* plays pivotal role in generation of diverse DC subtypes. The development of $CD8\alpha^+$DC and pDC requires *Irf8*, whereas $CD4^+$DC subset is dependent on *Irf4*. Our major area of focus is to understand the mechanisms of IRF directed diverse DC subtype development and understanding the cross talk of IRFs with other transcription factors and signaling pathways.

TGF-β/BMP signaling has pleiotropic effects on immune cell development and functions. While, analyzing the effect of *Irf8* expression on TGF-β superfamily, using a commercially available TGF-β/BMP pathway focused PCR array based approach, we had identified the *Acvrl1* gene (*Alk1*, a type I receptor of TGF-β superfamily), highly induced by *Irf8* in DCs. Further, through analysis of gene expression pattern from purified splenic DC subtype populations and mice carrying homozygous BXH-2 ($Irf8^{R294C}$) mutation that selectively blocks development of $CD8\alpha^+$DCs; we demonstrated that *Acvrl1* is expressed specifically in $CD8\alpha^+$DC subtype. Co-expression of *Irf8* and *Acvrl1* or its physiological ligand *Bmp9* led to synergistic increase in $CD8\alpha^+$DCs. Towards understanding the regulation of *Acvrl1* transcription in murine DCs, we analyzed human and mouse *Acvrl1* gene using Ensembl genome browser (Fig. 1A)

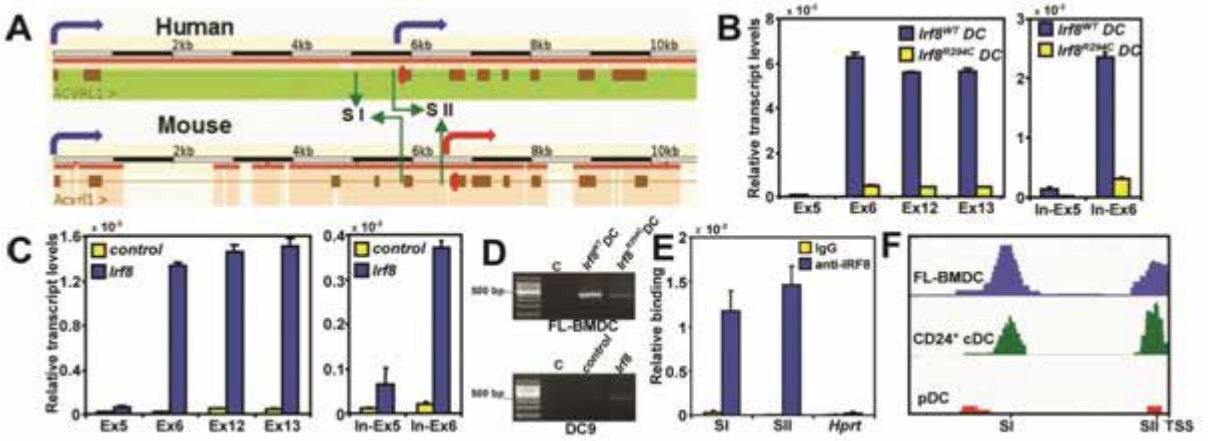

Figure 1: *Irf8* directly regulates *Acvrl1* gene expression in DCs
(A) Schematic diagram shows organization of human and mouse exons in *Acvrl1* gene (Shown up to ~10 kB). Blue arrows shows known and red arrows shows proposed TSS in mouse. Translation site is known as red bars. Exon (Ex) and Intron-Exon boundary (In-Ex) specific Q-PCR analysis of *Acvrl1* gene transcription from **(B)** $Irf8^{R294C}$ DC and control $Irf8^{WT}$ DC mice and **(C)** from control and *Irf8* expressing DC9 cells also correlates with proposed TSS. Results are representative of two independent experiments. Ex12 and Ex13 were analyzed by Q-PCR as control only to check the continuity of transcript. **(D)** 5' RACE analysis showed expected amplicon form $Irf8^{WT}$ DC whereas $Irf8^{R294C}$ DC shows very faint band correlating with very low transcription. Expected PCR amplicon can be seen in DC9 cells upon *Irf8* expression (lower panel). **(E)** ChIP assay from FL-BMDCs confirm recruitment of IRF8 to the proposed binding sites. Results are representative of three independent experiments. **(F)** IRF8 binding sites (SI and SII) can also be seen as minor peaks in *Acvrl1* gene from recently reported IRF8 ChIP-sequencing studies. Error bars in graphs represents ± SD.

Based on exon specific Q-PCR analysis, we predicted transcription start site (TSS) upstream of exon 6 in murine DCs that co-related with previously reported human ACVRL1 transcript starting from Exon 3 (Fig. 1B and C). TSS upstream of Exon 6 is also supported by Intron-Exon boundary specific Q-PCR transcript analysis. Proposed TSS was also confirmed by 5' RACE analysis in murine DCs (Fig. 1D) and it correlated with dominant TSS seen in CAGE-TSS data from FANTOM5 promoterome collection. We identified two IRF8 binding sequences upstream of major TSS in mouse *Acvrl1* gene and confirmed IRF8 binding to these sites by ChIP assay (Fig. 1E). IRF8 binding sites confirmed by ChIP analysis can also be seen as peaks upstream of TSS in recently reported IRF8 ChIP-sequencing studies (Fig. 1F). IRF8 is selectively recruited near major TSS of *Acvrl1* in CD8α⁺DC equivalent CD24⁺cDC but not in pDC subtype (Fig. 1F). Our study demonstrated that IRF8 directly regulates *Acvrl1* gene expression in DCs and further suggests that CD8α⁺DC specific factors help differential recruitment of IRF8 near major TSS of *Acvrl1* gene and thus explains selective expression of *Acvrl1* in CD8α⁺DC as opposed to pDCs. Future studies with emphasis on identification of CD8α⁺DC specific factors that aids IRF8 recruitment would help to better understand the regulation of *Acvrl1* gene expression in DCs.

Towards the physiological understanding of ACVRL1 signaling in DC development, we had shown that BMP9 supplementation of FL-BMDC cultures led to a selective increase in CD8α⁺DC development and suppression of pDCs. Recent report suggested that BMP10, another high affinity ligand for ACVRL1 can be present in active form in serum. Supplementation of BMP10 also led to selective increase in CD8α⁺DC development and suppression of pDCs as observed in BMP9 supplemented cultures. BMP signaling results in the phosphorylation of SMAD1/5/8 against the phosphorylation of SMAD2 resulting from TGF-β signaling. BMP9 signaling in FL-BMDCs led to selective and efficient phosphorylation of SMAD1/5/8, indicating that ACVRL1 signaling is active during DC development. *Id2* plays a pivotal role in DC subtype

differentiation; enforced expression of *Id2* specifically blocks pDC development whereas it is essential for the CD8α⁺DC development. BMP9 stimulation of FL-BMDCs also induced *Id2* gene transcription at early stage (2 h); though $Irf8^{R294C}$ DCs showed low levels of BMP9 stimulated *Id2* gene induction due to defect in *Acvrl1* receptor expression. BMP9 stimulation of FL-BMDCs specifically induced *Id2* gene and this was efficiently blocked by presence of ACVRL1-Fc in culture; whereas pDC and CD8α⁺DC subtype specific transcripts remained unaffected. These results implies that observed effect of BMP9 were specifically mediated by signaling through ACVRL1 receptor and suggests an explanation for observed tilt in DC differentiation towards CD8α⁺DC subtype. Together, we demonstrated that among different DC subtypes *Acvrl1* gene is differentially expressed in CD8α⁺DC (or its equivalent DC population) and ACVRL1 signaling contributes to DC diversity generation by specifically enhancing CD8α⁺DC development and suppressing that of pDCs. This finding is published in a Cutting Edge section of Journal of Immunology.

Publication

Original peer-reviewed article

1. Verma R, Jaiswal H, Chauhan KS, Kaushik M, Tailor P (2016) Cutting Edge: ACVRL1 signaling Augments CD8α⁺ dendritic cell development. *J Immunol* **197**: 1029-1034.

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Biology of T lymphocytes

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

In vivo T cell activation normally takes place following presentation of antigen by professional antigen-presenting cells. We used bone-marrow derived dendritic cells [BM-DCs] pulsed with Ova-II peptide to stimulate naive OT-II cells *in vitro* to mimic the *in vivo* priming situation. Interestingly OT-II cells activated by peptide-pulsed DCs at 40°C showed more Th1-dominant outcome, than that seen at 37°C incubation; and unlike that seen with activation of naive CD4 T cells by plate coated anti-CD3 and anti-CD28 antibodies in the absence of DCs. This could be attributed to an early induction of IL-12 by DCs at 40°C in presence of T cells, but not in their absence, as measured by IL-12 p40 mRNA detection. Addition of TRPV1 agonist capsaicin in the T cells:DC cultures at 37°C reversed this pattern and OT-II cells activated in presence of capsaicin showed a more pronounced Th2 differentiation with higher production of IL-4 and IL-13. Molecular interactions involved in this process need further characterisation.

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

To predominantly understand the genetic contribution to the immune cell phenotype in humans blood samples from siblings were collected. Data showed that in certain cell subsets differences in the frequencies within sibling pairs were less and those between unrelated donors was more and this difference was statistically significant. Thus, the difference in the frequencies of total T cells, CD4 memory cells, gamma-delta T cells, total monocytes, classical monocytes, patrolling monocytes, myeloid DCs and plasmacytoid DCs between sibling pairs and non-siblings was statistically significant ($p < 0.05$, $n>20$). In contrast such a difference was not

observed in CD8 memory cells, NK cells, NK T cells and plasmablasts. These data further highlight that the niche sizes for certain immune cell subsets are likely to be genetically regulated.

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

Mice were given 2 % DSS in water for 5 days, followed by normal water and morbidity and mortality was observed along with weight loss. In about 10 days from the initiation of DSS treatment wild type C57Bl. 6 mice succumbed to colitis. In contrast TAP1-null mice and beige mice showed resistance. As a follow-up on earlier observations showing CD80 expression contributing to the susceptibility to DSS-induced colitis and CD80-null mice showing resistance we made bone-marrow chimeric mice following lethal radiation. BM cells from CD80-null and wild-type mice were transferred to both CD80-null (CD45.2) and congenic wild-type (CD45.1) irradiated mice making 4-way chimeras. After an 8-10 week reconstitution period these mice were tested for chimerism. When tested for DSS-induced colitis, interestingly, CD80-null-to-wild-type and wild-type-to-CD80-null mice were found to be equally susceptible to morbidity and mortality. These preliminary data suggest that presence of CD80 on radiation-resistant cells of the mucosal lining as well as BM-derived cells in the mucosa is detrimental and leads to colitis following DSS treatment.

Publications

Original peer-reviewed articles

1. Jain N, Khullar B, Oswal N, Banoth B, Joshi P, Ravindran B, Panda S, Basak S, George A, Rath S, Bal V, Sopory S (2016) TLR-mediated albuminuria needs TNFα-mediated co-operativity between TLRs present in hematopoietic tissues and CD80 present on non-hematopoietic tissues. *Dis Model Mech* **9**: 707- 717.
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3. Prabhu SB, Rathore DK, Nair D, Chaudhary A, Raza S, Kanodia P, Sopory S, George A, Rath S, Bal V, Tripathi R, Ramji S, Batra A, Aggarwal KC, Chellani HK, Arya S, Agarwal N, Mehta U, Natchu UC, Wadhwa N, Bhatnagar S (2016) Comparison of human neonatal and adult blood leukocyte subset composition phenotypes. *PLoS One* **11**: e0162242.
4. Balyan R, Gund R, Ebenezer C, Khalsa JK, Verghese DA, Krishnamurthy T, George A, Bal V, Rath S, Chaudhry A (2017) Modulation of naïve CD8 T cell response features by ligand density, affinity and continued signaling via internalized TCRs. *J Immunol* **198**: 1823-1837.
5. Jain N, Oswal N, Chawla AS, Agrawal T, Biswas N, Vrati S, Rath S, George A, Bal V, Medigeshi GR (2017) CD8 T cells protect adult naive mice from JEV-induced morbidity via lytic function. *PLoS Negl Trop Dis* **11**: e0005329.

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Plasmodium proteins involved in virulence and host modulation: Host-parasite interactions in plasmodium liver stages

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Plasmodium species introduce effector molecules into hepatocyte 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 current projects aim to identify parasite proteins that play role in liver stage parasite development. The parasite proteins can be evaluated for vaccine potential, provided they could be expressed as recombinant proteins, or as drug targets. We also study parasite proteins likely involved in manipulation of host immune responses.

Vaccine potential of a Malaria DNA-J domain containing protein

PBANKA_1238200 codes for a 533 amino acid long 61496 Da protein which contains a conserved DNAJ domain and we have named it as Pb-DJCP 61.5. This protein is conserved across *Plasmodium* species. Our bioinformatics analysis shows that this protein is primarily helical in nature (18 helices and two beta sheets). Experimental (CD) data obtained by us, using the purified protein, lends support to the secondary structure prediction. The N-terminal 340 amino-acids (a.a.19-359) was expressed in *E.coli* and affinity purified. Recombinant protein when used for mouse immunization, it induced a very strong antibody response (~3 million) indicating its highly antigenic nature. Immune mice when challenged with 10000 sporozoites (over 20 fold in excess of natural infection dose) showed a 4-log scale reduction in parasite burden thus showing vaccine potential.

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

Longitudinal evaluation of CD4+FoxP3+ T-reg cell levels in a human sporozoite challenge study documented that an increased number of these cells was associated with higher parasite loads and a decline in pro-inflammatory cytokines. Using bioinformatics based analysis we have identified a putative T-cell modulatory protein from malaria parasites. We have characterized the transcript and protein profile across the life stages of the parasite. We have also generated a clonal population of a parasite lacking this gene coding for the Tip protein.

Screening of Kinase inhibitor library against *P. berghei* FIKK kinase (MLFK)

We have shown that a novel FIKK kinase (MLFK) regulates the development of mosquito and liver stages of the malaria. [Scientific Reports 6, 10.1038/srep39285]. To find an inhibitor against MLFK, we screened the library from GSK (PKIS-1). Out of 357 kinase inhibitors tested seven showed in the range of 50 to 72 % inhibition at the highest dose (100 μM). None were very promising hence more screening is required to get a good inhibitor.

Screening of *Candida* Hyphae formation inhibitors

The process through which *Candida albicans* form hyphae is complex. Several work has been done to find out the genes responsible for the hyphae formation and adherence. We screened a range of commercially available organic compounds and zeroed on a compound which inhibit hyphae formation at very low dosage. We currently term it as ARSAP-101 and it completely inhibited hyphae formation at a low dose of <0.5% (v/v) in cell culture conditions. ARSAP-101 is non toxic to mouse at 5% (v/v) dose given in the drinking water for more than 4 days.

Publication

Original peer-reviewed article

1. Jaijyan DK, Verma PK, Singh AP (2016) A novel FIKK kinase regulates the development of mosquito and liver stages of the malaria. *Sci Rep* **6**: 39285.

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Genetic and functional analyses of host and HIV-1 genes that affect pathogenesis

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Pathogens make use of the host cell machinery to establish, proliferate and cause the disease. This is true for many bacterial, viral and parasitic diseases that affect humans. The major theme of our research is to understand how viruses exploit the host cell machinery for its own advantage using several interrelated approaches.

mTORC1 modulates HIV-1 replication and controls latency

HIV-1 efficiently redirects/reprograms various cellular pathways to meet its requirements. We hypothesized that mTORC1 being a central regulator of biosynthesis may be exploited by HIV-1. Indeed, our experiments show that mTORC1 is hyperactivated at 12 and 24 hours post infection.

We further wanted to know if mTORC1 is involved in breaking the latency in HIV-1. We tested its role in two cell line- based latency models (U1 monocytic model and J1.1- T cells). They harbour integrated HIV-1 provirus and when stimulated by PMA or TNF-a, produce large amounts of virus. Specific inhibition of mTORC1 by inhibitor TORIN inhibited viral reactivation. Altogether, this study establishes the important role of mTORC1 signalling in the life cycle of HIV-1 and how one regulatory protein Tat alone is sufficient to influence this pathway for its own propagation.

Role of De-ubiquitination (DUB) in HIV-1 biology

It is now increasingly recognized that DUBs also play a very critical role in several complex cellular processes. We observed that one of the major DUBs, USP7, plays a critical role in stabilizing HIV-1 Tat protein. USP7 specific as well general DUB inhibitors reversed this process.

Growth factor deprivation/serum starvation leads to reactivation of HIV-1 in latently infected monocytes

We found that growth factor deprivation breaks latency in monocytes. We also tested other crucial factors like NF-KB, SP1 and AKT and found that they do not regulate viral reactivation. Our preliminary report suggests that growth factor deprivation up-regulates ERK/JNK pathway which then leads to phosphorylation of c-Jun and this plays an important role in viral reactivation. Treatment of cells with U0126 (ERK/JNK pathway inhibitor) in such conditions inhibited viral replication.

Curcumuin inhibits HIV-1 replication by targeting Tat protein

Unfolded or disordered proteins are degraded by 20S proteasome in an ubiquitin independent manner. Since HIV-1 Tat protein is largely unstructured, we hypothesized that Tat may be targeted by this pathway. Tat-transfected cells when treated with curcumin resulted in time and dose-dependent degradation of Tat protein.

Modulation of HIV-1 pathogenesis by TB and Other viruses

Majority of HIV-1 infected individuals are also infected with TB and other viral diseases in India. TB, Dengue, Hepatitis C virus co-infections are quite common. We wish to characterize exosomes from infected in vitro culture supernatants and also from patients serum for possible biomarkers (miRNA, impact on innate immunity etc).

Publications

Original peer-reviewed articles

1. Kumar B, Arora S, Ahmed S, Banerjea AC (2017) Hyperactivation of mammalian target of rapamycin complex 1 by HIV-1 is necessary for virion production and latent viral reactivation. *FASEB J.* **31**:180-191.
2. Ali A, Banerjea AC (2016) Curcumin inhibits HIV-1 by promoting Tat protein degradation. *Sci Rep.* **6**:27539.
3. Ali A, Raja R, Farooqui SR, Ahmad S, Banerjea AC (2017) USP7 deubiquitinase controls HIV-1 production by stabilizing Tat protein. *Biochem J.* **474**:1653-1668 v

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Study of mucosal immune responses

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IgM memory, long overlooked, has recently been identified as the major fraction that can contribute to germinal center reactions in secondary responses, but murine IgM memory has been difficult to characterize phenotypically as the cells do not express specific markers, such as CD27 that has been used to identify human memory B cells. We determined whether differential expression of the markers CD73, CD80 and CD273, which have been used to characteize less-mature and more-mature isotype-switched memory B cell populations, can be used to identify IgM memory cell subsets formed in response to environmental antigens in unmanipulated mice as arising from T cell-dependent or T cell-independent responses. We found that the majority of IgM memory cells in WT mice expressed either CD73, CD80 or CD273 (singly or in combination). This number was significantly lower in mice lacking T cell help or CD40, with a major deficit seen in the $CD73^+$ subset. Mixed bone marrow chimeras between WT/ TCR-null and WT/CD40-null mice revealed that the subset was rescued in the TCR-null donor pool, but not in the CD40-null donor pool. CD40-deficiency did not affect CD73 expression on other cells such as neutrophils, B-1 B cells, marginal zone B cells, and regulatory T and B cells. However CD73 expression was lower on CD4 and CD8 T cells and on age-associated B cells. Thus, in addition to its reported effects on B cell differentiation, CD40-signaling can influence the constitution of the memory B cell pool and may also promote controlled T cell responses *in vivo*.

We have also been looking at the effect of host genotype on maintenance of the microbe-immune homeostatis in the gut. We found alterations in fecal bacterial diversity in a variety of single gene-deficient mouse strains

(beige, CD80$^{-/-}$ ICAM-1$^{-/-}$ MyD88$^{-/-}$, TCRδ$^{-/-}$ TCRβ$^{-/-}$ CD40$^{-/-}$ and VDR$^{-/-}$). Interestingly, all knockout strains showed a greater abundance of Proteobacteria, which have been shown by other investigators to cause gut inflammation. As these bacteria are targeted by IgA arising largely from T-independent responses, the results indicated that a possible link between host genotype and the quality and/or quantity of IgA. We found that commonly used wild-type strains differed in their ability to make IgA, and this correlated with the proportion of bacteria in their fecal pellets that were coated with IgA. High IgA-coating of bacteria correlated negatively with fecal bacterial loads, in keeping with a role for IgA in containing intestinal bacteria and preventing their systemic entry. The representation of specific bacterial taxa also differed between the strains, with Firmicutes being over-represented in Iga-low mice and Bacteriodetes being over-represented in IgA-high mice. Higher Firmicutes:Bacteroidetes ratios have been associated with aging, inflammation and metabolic disorders and we found that the IgA-low mice were more susceptible to experimental colitis evern thought they were disease-free under normal conditions. Together, our data indicate that host genotype and adaptive immune components determine the nature of intestinal homeostasis and the ease with which it can be disrupted following injury to the epithelial barrier.

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Analysis of *Salmonella* - host cell interactions

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Pathogenic *Salmonella* species continue to be a major public health problem in the developing world. Different *Salmonella* serovars produce different clinical manifestations in different hosts. *Salmonella typhi* (*S.typhi*), causes systemic infection, typhoid, in humans while non-typhoidal *Salmonella* serovar, *Salmonella typhimurium* (*S.typhimurium*) produces only self-limiting localized gastroenteritis. Significantly, *S.typhi* does not establish infection in mice whereas *S.typhimurium* infection in susceptible strains of mice results in a systemic manifestation that is analogous to human typhoid. The reasons for different clinical outcomes produced by *S.typhi* and *S.typhimurium*, and for the host specificity exhibited by these two closely related *Salmonella* serovars are not understood. We have been studying differences in the interactions of *S.typhi* and *S.typhimurium* with host cells, and investigating modulations of immune responses by pathogenic *Salmonella* that enable it to establish infection.

Caspase-1 generated stimulus promotes *Salmonella* invasion

Macrophages sense pathogens and other kinds of stresses through intracellular Nod-like receptors (NLRs). Activation of the Nlrc4 inflammasome by intracellularly delivered flagellin monomers (and other virulence factors) produced by *Salmonella* brings about macrophage cell death accompanied by release of IL-1β and IL-18 collectively referred to as pyroptosis. This pyroptotic cell death serves as a major mechanism of bacterial clearance during experimental infection of mice with *Salmonella typhimurium*. Recent studies have demonstrated that caspase-1 also gets activated during infection of intestinal epithelial cells (IECs) with *Salmonella* and plays an important role in clearing the

pathogen from the gut. We have previously reported an interesting phenomenon wherein caspase-1 – derived lipid stimulus regulates expression of flagellin during infection of macrophages with *Salmonella* and serves as an amplification loop for pyroptosis. Further experiments showed that cues derived from casapse-1 might imprint upon bacteria the capability to replicate better in nutrient poor atmosphere that prevails inside *Salmonella*-containing vacuoles in macrophages. *Salmonella* isolated from WT macrophages also had higher levels of ATP as compared to bacteria isolated from caspase-1 deficient macrophages. We now show that caspase-1 – generated stimulus also triggers production of molecules from *Salmonella* that are known to play a critical role in the invasion of epithelial cells with this pathogen. Treatment with the stimulus derived from *Salmonella* – infected caspase-1-expressing but not caspase-1 deficient macrophages resulted in increased production of *Salmonella* invasion promoting molecules, SipA and SipC. These molecules are known to participate in triggering actin cytoskeletal rearrangements during invasion of IECs with *Salmonella*. The increase in the expression of Sips was associated with increase in the ability of *Salmonella* to invade intestinal epithelial cells. Significantly, caspase-1 – dependent promotion of invasion capability was also seen during infection of intestinal epithelial cells with *Salmonella*. Our findings suggest that *Salmonella* might exploit cues generated by caspase-1 activation for promoting cellular invasion.

Immunity - relevant B-cell responses against *Salmonella typhi* show negligible cross-reactivity with closely related *Salmonella* serovar, *Salmonella typhimurium*

Salmonella typhi and *Salmonella typhimurium* produce different clinical manifestations in humans in spite of sharing a high degree of homology at the genome level. Immunization with live oral vaccine *S.typhi* Ty21a does not offer protection against infection with *S.typhimurium*. To understand reasons for this inability, we demonstrated previously that administration of live *S.typhi* to mice generates a robust antibody response against cell surface associated antigens including O-antigenic determinants of LPS. Interestingly, while these antibodies showed cross-reactivity with LPS from *S.typhimurium* in ELISA, these did not bind *S.typhimurium* when analyzed by flow cytometry, suggesting that shared O-antigenic determinants may not be exposed on the cell surface and therefore may not be accessible for binding to the antibody. We now report that *S.typhi*-immune mice also produced antibodies against several cell wall associated protein antigens shared by *S.typhi* and *S.typhimurium*, as determined by Western blotting. Yet, the immune sera showed negligible reactivity with live/intact *S.typhimurium* when compared with *S.typhi*. Cross - reactivity was also observed at the level of T cells as splenocytes from *S.typhi*-immune mice readily produced IFN-γ upon *ex vivo* stimulation with antigens from *S.typhimurium*. However, in spite of this cross-reactivity, these mice did not survive long when challenged with *S.typhimurium* highlighting a critical role for antibodies in preventing infection with *Salmonella*. Using *in vitro* models of infection, we show that these serovar - specific antibodies are capable of inhibiting invasion of human epithelial cells with *Salmonella* in a serovar-specific manner. These antibodies also enhanced phagocytosis and intracellular clearance of *Salmonella* by macrophages. Our findings reveal previously unappreciated mode and degree of serovar specificity in B cell responses relevant to immunity against *Salmonella* infection.

Inflammatory responses during infection with *Salmonella*

TLRs and more ---

During infection with pathogenic *Salmonella*, the inflammatory and innate immune responses are initiated through activation of pattern recognition receptors including

TLRs and NLRs. *Salmonella* expresses several conserved molecular patterns most notably LPS and flagellin, which are recognized by TLR4 and TLR5 respectively. Invasive *Salmonella* species also produce virulence factors encoded by several pathogenicity islands (SPI) which are critical for invading intestinal epithelial cells and inducing inflammatory cytokines and chemokines from these cells. In experimental models of *Salmonella* infection, TLRs play a major role in generating inflammatory responses. However, since LPS and flagellin are also present in non-invasive avirulent bacteria, their involvement in pathogenic context has not been well studied. Are these two molecules released in sufficient amounts to generate (either alone or in combination) inflammatory responses of the magnitude observed with live bacteria, or are the inflammatory responses an outcome of cross-talk between pathways engaged by virulence factors and those activated by the TLRs? We show that intraperitoneal infection of C57BL/6 mice with *Salmonella* generates inflammatory cytokines with live but not antibiotic-treated bacteria, suggesting requirement for metabolically active pathogen in the induction of these responses. The amount of LPS present in the bacterial inoculum that was used for infection did not trigger any detectable inflammatory response *in vivo*. Interestingly, infection of MyD88 (TLR adaptor) deficient mice with *Salmonella* generated cytokine responses which were comparable with those produced in WT mice at early time point but significantly reduced at later time points suggesting involvement of non-TLR pathway(s) in the initial phase of infection. These findings provide basis for further experimentation to get better insights into the induction of innate and inflammatory responses during infection with pathogenic *Salmonella*.

Publication

Original peer-reviewed article

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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) how these virulence factors interact with the immune system and host cell to alter its cellular and molecular processes, and (c) evaluating the vaccine potential of pneumococcal surface antigens.

Extracellular vesicle associated endo-deoxyribonuclease from *S. pneumoniae* degrades neutrophil extracellular traps

Pneumococci are cleared from the host primarily by antibody dependent opsonophagocytosis by phagocytes like neutrophils. Neutrophils release neutrophil extracellular traps (NETs) on contacting pneumococci. NETs immobilize pneumococci and restrict its dissemination in the host. Pneumococci utilize various strategies to evade the host immune response. One such strategy involves the release of deoxyribonuclease(s) [DNase(s)] to degrade NETs. To identify novel DNase(s), the secretome of autolysin-deficient *S. pneumoniae* was screened for DNase activity. In-gel assay done using secretome revealed 3 bands with DNase activity. Mass spectrometric and bioinformatic analyses led to the identification of SPD_1788 as a potential DNase. The ORF encoding SPD_1788 was cloned, expressed and the recombinant protein purified to homogeneity from *E. coli* inclusion bodies. Unlike most nucleases, SPD_1788 showed activity even in the absence of divalent cations. The activity was enhanced in their presence. SPD_1788 digested supercoiled plasmid DNA suggesting that it is an endodeoxyribonuclease. Confocal microscopy showed that pneumococcal secretome degraded NETs released from activated human neutrophils. SPD_1788 is associated with extracellular vesicles

(EVs). EVs from wildtype pneumococci degraded NETs whereas EVs from SPD_1788 deficient pneumococci showed little NET degrading activity. Recombinant SPD_1788 efficiently degraded NETs. NET degradation by SPD_1788 deficient pneumococci was compromised compared to the wildtype strain. SPD_1788 deficient strain exhibited decreased bacterial load, lung pathology and virulence in a murine-sepsis model compared to the wildtype strain. This study provides insights into the role of a novel EV associated endodeoxyribonuclease in evasion of the innate immune system.

Characterization of purine nucleoside phosphorylases from *S. pneumoniae*

Nucleotide metabolism is important as nucleotides are essential for a wide variety of bacterial processes, and are synthesized by *de novo* and salvage pathways. Purine nucleoside phosphorylase (PNP) is a key enzyme involved in salvage pathway of purine biosynthesis. PNP catalyses the cleavage of the glycosidic bond of ribo and deoxyribo nucleosides in the presence of inorganic orthophosphate to produce a purine base and ribose or deoxyribose-1-phosphate. Intriguingly *S. pneumoniae* is predicted to encode two proteins (SPD_0726 and SPD_0730) with potential PNP activity. We are interested in biochemically characterizing the two PNPs and deciphering their potential role in host-pathogen interaction.

Our bioinformatic analysis revealed that SPD_0726 and SPD_0730 were present in all the 29 strains for which complete genome sequence is available thereby suggesting that PNPs play an important role in the biology of *S. pneumoniae*. SPD_0726 and SPD_0730 share 15.1 % identity at the amino acid sequence level. SPD_0726 and SPD_0730 were highly conserved. The ORFs coding for SPD_0726 and SPD_0730 were cloned in an *E. coli* expression vector and the recombinant proteins were purified to homogeneity using affinity chromatography. Purine nucleosides adenosine, 2'-deoxyadenosine, guanosine, 2'-deoxyguanosine, inosine and 2'-deoxyinosine, and pyrimidine nucleosides cytidine, uridine and thymidine were used as potential substrates to determine the substrate specificity of SPD_0726 and SPD_0730. The kinetic parameters K_m, V_{max} and catalytic efficiency were determined for the two enzymes for each substrate. Our studies indicate that the preferred substrates of SPD_0726 were guanosine, 2'-deoxyguanosine and inosine. Adenosine, 2'-deoxyadenosine, guanosine and inosine were the preferred substrates for SPD_0730. The *in vitro* growth of SPD_0726 and SPD_0730 deficient mutants was highly compromised compared to the wildtype strain. Our study is likely to provide new insights regarding pneumococcal nucleotide metabolism and may form the basis for designing novel drugs against *S. pneumoniae*.

Patent

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

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The major theme of research is to evaluate polymeric particle based delivery systems for improved immunogenicity of different antigens such as Tetanus Toxoid (TT), Hepatitis B surface antigen (HBsAg), viral and carbohydrate (Vi polysaccharide and *Streptococcus pneumoniae* polysaccharides) based vaccines. Another major research activity of the laboratory is the analysis of inclusion body formation and development of mild solubilization processes for improved recovery of bioactive proteins.

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. Researches in the following areas are conducted in the laboratory to achieve the following objectives:

A. Immunogenicity of carbohydrate antigens entrapped in polymer particles

Polymeric particles entrapping protein/ carbohydrate antigens are being routinely used in the laboratory to improve their immunogenicity. It was observed that both pneumococcal polysaccharides and Vi polysaccharides entrapped in nanoparticles induce memory antibody response and promoted isotype switching from single dose immunization. Major emphasis of the laboratory is now to develop nanoparticle based pneumococcal vaccine.

I. Formulation and evaluation of pneumococcal vaccine using polymeric nanoparticles

It was observed that pneumococcal capsular polysaccharide entrapped in PLA

nanoparticles 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 these capsular polysaccharides to see if protective immunity can be achieved using polymeric delivery systems. Recently SP0845 protein of *S. pneumoniae* was reported to provide protection against pneumococcal infection. We are exploring to make conjugate vaccine encompassing polysaccharide and SP0845. It is expected that these conjugates will provide better protection in terms of antibody generation and opsonophagocytic assay.

II. Formulation and evaluation of dry powder alum as an adjuvant

Alum gel was re-formulated to form a dry powder using spray drying. Unlike gel alum which formed irreversible aggregates upon freezing, alum particles did not lose their structure on being exposed to freezing conditions. It was of interest to know if alum particles could retain their adjuvanticity during freezing. For this, alum particles were stored at -70°C and later DT (Diphtheria Toxoid) was adsorbed on to them. It was observed that alum particle stored at -70°C have similar adjuvant properties as gel alum. Spray dried alum particles adsorbing antigens were also developed to formulate temperature stable vaccines. Powder form of vaccine preparation comprising of TT and alum when stored at -70°C and immunized also elicited comparable antibody titers. The efficacy of this dry powder alum formulation as an adjuvant and its stability at subzero temperatures offer new possibilities of developing temperature resistant alum based vaccine formulations.

B. Solubilization and refolding of inclusion body proteins

Mild solubilization of inclusion body proteins without using high concentration of chaotropes results in high throughput recovery of bio-active proteins. Attempts were made to develop of a suitable buffer cocktail for solubilization of inclusion body aggregates for improved recovery of bioactive protein. Different combination of mild solubilization buffer such as propanol, pH 12 with 2 M urea, 6 M trifluroethanol (TFE) were used to develop a buffer for solubilization of inclusion body aggregates.

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Original peer-reviewed articles

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Patent

1. Panda AK, Kanchan V (2016) Polymer particles based vaccine (A vaccine composition comprising polymeric nanoparticles and microparticles for inducing TH1 and TH2 response (*Indian patent no. IN278769 granted on 30/12/2016*).

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Disorders of proliferation: Analysis of novel pathways and targets

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Tumor-derived hCG is often associated with poor patient prognosis. hCG mediates pro-proliferative effects on tumor cells, in addition to inducing the generation of inflammatory, angiogenic, pro-invasive and immunosuppressive mediators; such effects arise as a consequence of direct effects of hCG on tumor cells or as a consequence of hCG-driven collaboration between tumor cells, host T cells and host monocytes.

Poor prognosis in cancer patients is often associated with chemoresistance. Pre-incubation of tumor cells with hCG induces resistance to several cytotoxic drugs. Of interest is the fact that LH cannot mediate similar chemo-protective effects. If tumor cells express receptors that can distinguish between these closely related hormones, it would put into some context the observations that aberrant expression of hCG, rather than of LH, is more frequently linked with tumor cell aggressiveness.

TLR ligands are gaining prominence as mediators of chemoresistance in tumor cells. Combined hCG and TLR ligand incubation on tumor cells induces synergistic chemoresistance. Apoptotic blebs arising upon the action of chemotherapeutic drugs on tumor cells contain endogenous TLR ligands. Whether such blebs too act in synergy with hCG to impart chemoresistance is an area of focus.

Upon the subcutaneous implantation of Lewis Lung Carcinoma (LLC) cells in female βhCG transgenic (C57BL/6 x $FVB^{\beta hCG/-}$ F1) mice, tumor incidence, and volume are heightened. In tumor tissue and in the ovaries (but not in splenocytes), enhanced transcription of molecules associated with angiogenesis, inflammation, metastasis and apoptosis is

observed. In ovariectomized transgenic mice, LLC cell implantation results in a reduction in the lag phase, and further increases in tumor incidence and tumor volume. Thus, hCG acts on tumor cells *in vivo* to induce significant pro-tumorigenic effects, an event that may be ameliorated by ovarian stimulation. From a physiological perspective, whether the high steroid levels in pregnancy negate the pro-tumorigenic effects of hCG is worth considering.

Sera derived from lupus patients exhibit IgG anti-Hb humoral autoreactivity. Hb induces the heightened secretion of lupus-associated cytokines from splenocytes derived from old lupus-prone mice; $CD4^+$T cells appear to be uniquely sensitive to the inflammatory effect of Hb *in vitro*, whereas B cells are non-responsive. Immunization of lupus-prone (but not healthy) mice with Hb leads to the generation of antibodies to several self-moieties and enhanced-onset glomerulonephritis. The work positions Hb both as an antigen and a pathology-perpetuating immunogen in a murine model of lupus. It exhibits the properties of an 'ideal' autoantigen: It is normally sequestered, is released as a consequence of disease processes and is consequently heightened in circulation, is inherently inflammatory, is endogenously immunogenic only in a lupus milieu, and finally is disease-enhancing upon immunization, again only in a lupus milieu. Whether anti-Hb antibody levels are of supplemental diagnostic and/or prognostic value in humans would be interesting to evaluate.

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Original peer-reviewed articles

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3. Malhotra SS, Baanerjee P, Chaudhary P, Pal R, Gupta SK (2017) Relevance of Wnt10b and activation of β-catenin/GCMa/syncytin-1 pathway in BeWo cell fusion. *Am J Reprod Immunol***. 78** DOI:10.1111/aji.12676.

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

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To investigate the protective efficacy of MIP immunization in live or killed form, through parenteral route / by aerosol route, against subsequent infection with *M. tuberculosis* in animal models. Study of immune response to *M.tb* in 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 mechanism of MIP mediated host immune activation.

A. Efficacy of MIP as a booster to BCG

BCG-MIP vaccination elicits Poly-functional T cells and confers protection in M.tb challenged mice.

Not only magnitude but also quality of T cell response plays important role in protection against tuberculosis. Further, we evaluated the quality of T cell immune response on per cell basis post M.tb infection and their association with protection. In lungs, immunized groups had higher percentage of M.tb specific 3+ (IL-2, IFN- γ, TNF-α) CD4+ T cells when compared to saline group. Percentage of 2+ (IFN- γ, TNF-α) and 1+ (IFN- γ) CD4+ T cells were higher in the lungs of 'BCG-MIP booster' group as compared to 'only BCG' group. Since we analyzed the lung immune response when active infection with M.tb was underway, we observed more effector like signature as there were predominantly 2+ (IFN- γ, TNF-α) CD4+ T cells along with substantial proportion of 1+ (IFN-γ) CD4+T cells. Mean fluorescence intensity of these cytokines in lungs was compared. 3+ (IFN- γ, TNF-α, IL-2) CD4+ T cells had higher MFI of IFN- γ and TNF-α followed by 2+ and 1+ CD4+ T cells. To get effective protection in the lung and airways a vaccine against TB should induce robust local lung immune response, in addition to

systemic protective response. Booster vaccination by MIP aerosol has induced effective immune response in both the compartments. In conclusion, our data demonstrate for the first time, potential application of MIP as a booster to BCG vaccine for efficient protection against tuberculosis. MIP booster by aerosol route induced Th1 and Th17 immune response in the lungs of infected animals along with poly-functional T cells with effector function. MIP being a non-pathogenic whole bacterial vaccine with unique immunomodulatory properties, could be very cost effective strategy for efficient control of tuberculosis.

B. Role of MIP in modulation of cell death pathways in M.tb infected macrophages, when administered as therapeutic i.e post M.tb infection

M.tb is well known to modulate host immune response at several points. Inhibition of phago-lysosome fusion in the infected macrophages is an important strategy which promotes its survival. Keeping in view the immunotherapeutic potential of MIP and the crucial role which autophagy plays in clearance of M.tb from the host, effect of MIP on autophagic process in macrophages and its role in phagosome maturation and phago-lysosome fusion in M.tb infected macrophages was studied. It was observed that MIP induced autophagy in M.tb infected macrophages. Level of LC3II, which marks the autophagosome formation and thus the induction of autophagy, was found to be significantly high in 'MIP treated M.tb infected macrophages' as compared to 'only M.tb infected' macrophages. Formation of autophagosome was also analyzed by ultrastructural analysis. Role of MIP induced autophagy in phagosome maturation was studied. Level of Rab 5 which is marker for early phagosome and Rab 7 which is present on late phagosomes was determined by immunostaining. It was observed that significant Rab 5 to Rab 7 conversion takes place in MIP infected macrophages while M.tb inhibited this conversion process. When M.tb infected cells were also co-infected with MIP, it was observed that MIP promotes maturation of M.tb containing phagosomes. To check if autophagy plays any role in Rab 5 to Rab 7 conversion, cells were treated with the autophagy inhibitor, 3-Methyl Adenine and then infected with M.tb followed by MIP infection. Rab 5 to Rab 7 conversion was found to be inhibited in these cells, indicating blockage of maturation of M.tb containing phagosomes. In MIP infected macrophages, autophagosome formation was followed by autophagosome-lysosome fusion and lysosomal degradation while M.tb induced autophagosome formation but it inhibited its fusion with lysosome and proteolysis.

Publication

Original peer-reviewed article

1. Saqib M, Khatri R, Singh B, Gupta A, Kumar A, Bhaskar S (2016) *Mycobacterium indicus pranii* as a booster vaccine enhances BCG induced immunity and confers higher protection in animal models of tuberculosis. *Tuberculosis (Edinb)* **101:** 164e173.

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Fine tuning of NF-κB signaling

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Concomitantly activated cell-signaling pathways often crosstalk with each other. In an integrative approach, which combines experimental and mathematical analyses, we characterize pathway crosstalks and examine their role in determining cellular responses with a particular focus on the pleiotropic NF-κB system. Using mouse models as well as patient-derived cells, we are investigating how pathway crosstalks modulate physiological processes, such as immune activation, and whether aberrant pathway crosstalks are associated with human ailments, including multiple myeloma. Recently, we identified a crossregulatory mechanism that suppresses trafficking of naïve lymphocytes into inflamed lymph nodes. Similarly, we unmasked amyeloma-associated autoregulatory NFκB pathway, which imparts resistance in cancerous cells to chemotherapeutic drugs.

Publications

Original peer-reviewed articles

1. Chatterjee B, Banoth B, Mukherjee T, Taye N, Vijayaragavan B, Chattopadhyay S, Gomes J, Basak S (2016) Late-phase synthesis of IκBα insulates the TLR4-activated canonical NF-κB pathway from noncanonical NF-κB signaling in macrophages. *Sci Signal* **9**: ra 120.
2. Roy P, Mukherjee T, Chatterjee B, Vijayaragavan B, Banoth B, Basak S (2016) An autoregulatory RelB:p50 NF-κB pathway perpetuates pro-survival TNF response in multiple myeloma. *Oncogene* **36**:1417-1429.
3. Jain N, Khullar B, Oswal N, Banoth B, Joshi P, Ravindran B, Panda S, Basak S, George A, Rath S, Bal V, Sopory S (2016) TLR-mediated albuminuria needs TNFα-mediated cooperativity between TLRs present in hematopoietic tissues and CD80 present on non-hematopoietic tissues in mice. *Dis Model Mech* **9**: 707-717.

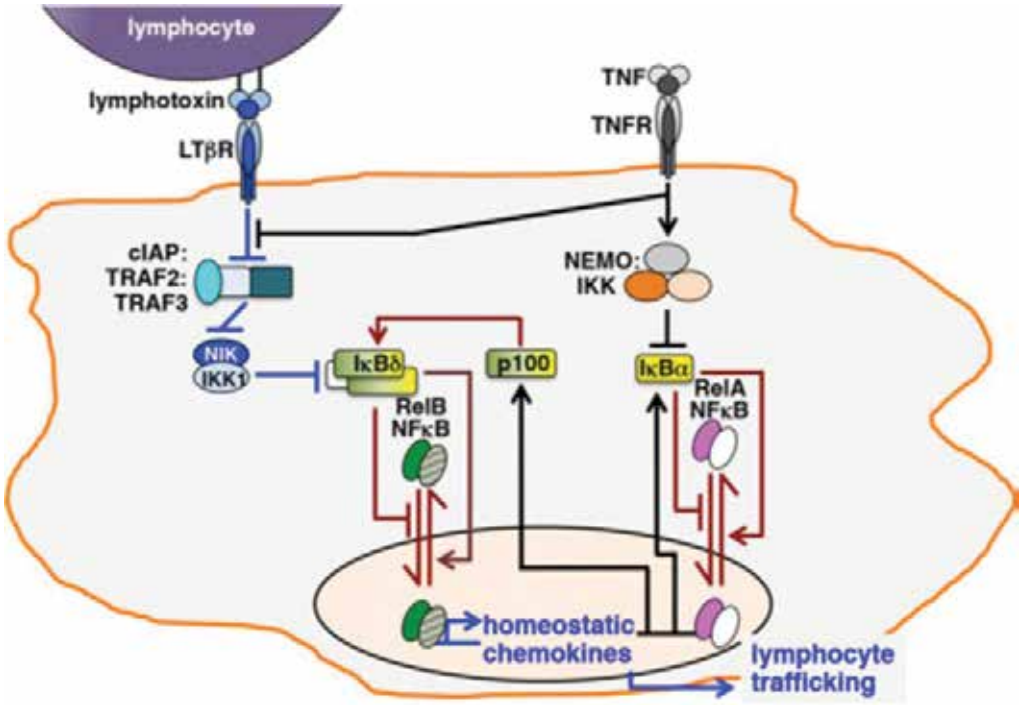

Figure. **A mechanistic model explaining TNF-mediated suppression of lymphocyte trafficking in inflamed lymph nodes:** In resting cells, TRAF2-TRAF3-cIAP complex promotes the degradation NIK, and p100-IκBδ retains RelB and other NF-κB monomers. Activation of LTβR leads to the proteolysis of TRAF2 and TRAF3 that rescues NIK from the constitutive degradation. NIK in association with IKK1 induces the processing of p100 into p52 that liberates the RelB heterodimers. TNF signal renders TRAF2 and TRAF3 resistant to degradation in LTβR-stimulated cells resulting in the inactivation of NIK. In addition, TNF-activated RelA dimers induce the transcription of *Nfkb2* mRNA, which encodes p100. Therefore, TNF treatment of LTβR-stimulated cells potently accumulates the precursor p100, which sequesters the pre-existing nuclear RelB dimers as inhibitory IκBδ and terminates RelB-mediated expressions of homeostatic chemokines. The proposed mechanism impedes continuing ingress of naïve lymphocytes in reactive secondary lymphoid organs.

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Vaccine Immunology: Harnessing follicular T helper cells for antiviral immunity

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Our research group aims to understand the biology and function of follicular T helper (TFH) cells in long-term protective immunity. TFH cells are the unique subset of CD4 helper T cells specialized in providing help to B-cells and controlling the germinal center (GC) derived antibody responses. By comprehensive monitoring and functional analysis of TFH cells in controlled human vaccination studies and animal models, we aim to decipher the positive attributes of TFH cells in constituting the long-lasting protective immunity.

In the current program, we are utilizing SA14-14-2 live attenuated Japanese encephalitis (JE) vaccine as the study model to investigate TFH cells. Live attenuated JE-vaccine is recommended for mass vaccination campaigns in India, which provides an opportunity to identify the TFH correlates of long-term protective antibody responses in human. Firstly, by employing genetically modified mice, we have validated that $CD4^+$ T-cells, but not $CD8^+$ T-cells, are implicated in the antibody response to the live attenuated JE-vaccine. By adoptively transferring the different subsets of $CD4^+$ T-cells from vaccinated mice into the TCR-β null mice, which lacks $CD4^+$ T-cells, we further confirms that only $CD4^+$ $CXCR5^+$ TFH-cell subset is responsible for the initiation of antibody response to JE-vaccine (Fig. 1A). The kinetics of TFH-cell development during the vaccine response shows significant correlation of the magnitude of TFH cell with the GC B-cell development. The data from our animal studies suggest that TFH cells play central role in formulating the germinal center derived antibody responses to the JE-vaccine. Further analysis on the serum samples from individuals immunized with the single dose of live attenuated JE-vaccine shows elevated serum levels of CXCL13 in individuals with high titer of vaccine-specific IgG (Fig. 1B). Because CXCL13 is the marker for TFH-cell coordinated activities in GCs, this data from human samples corroborates with our finding in animal models. Further studies are in line to determine if there is a unique TFH-cell subset preferentially induced by live attenuated JE-vaccine that formulates long-lasting protective antibody responses.

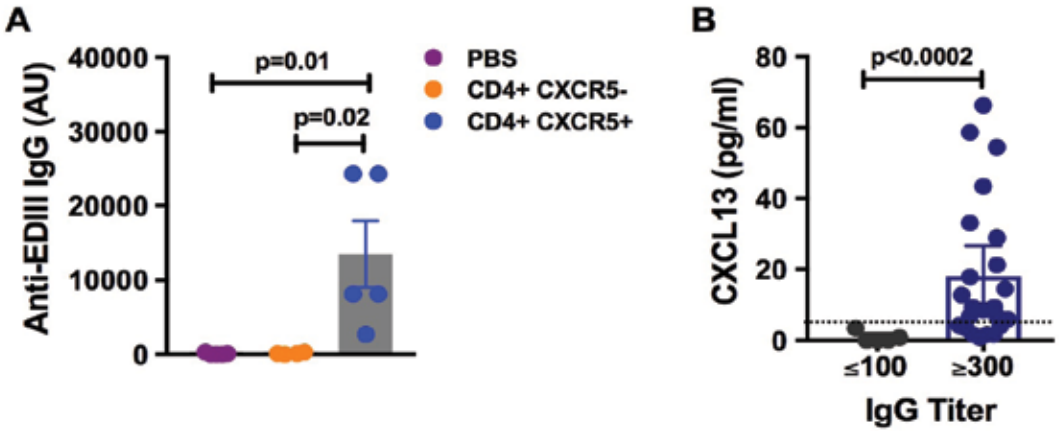

Figure 1. Follicular T helper cells are indispensable for antibody responses to live attenuated JE-vaccine. (**A**) IgG titer in different groups of TCR-β-null mice either adoptively transferred with the sorted TFH cells and non-TFH cells from vaccinated mice or with PBS as vehicle. Y-axis represents anti-EDIII IgG titer determined by ELISA. Statistics: one-way ANOVA followed by Bonferroni's multiple comparison test. (**B**) Serum CXCL13 levels in individuals (n=40) responding with low (≤100) or high IgG titer (≥300) to the single dose of live attenuated JE-vaccine. Y-axis represents concentration of CXCL13 determined by ELISA. Dotted line indicates the limit of detection 4 pg/ml. Statistics: Mann-Whitney non-parametric *t*-test.

Publication

Reviews/Proceedings

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

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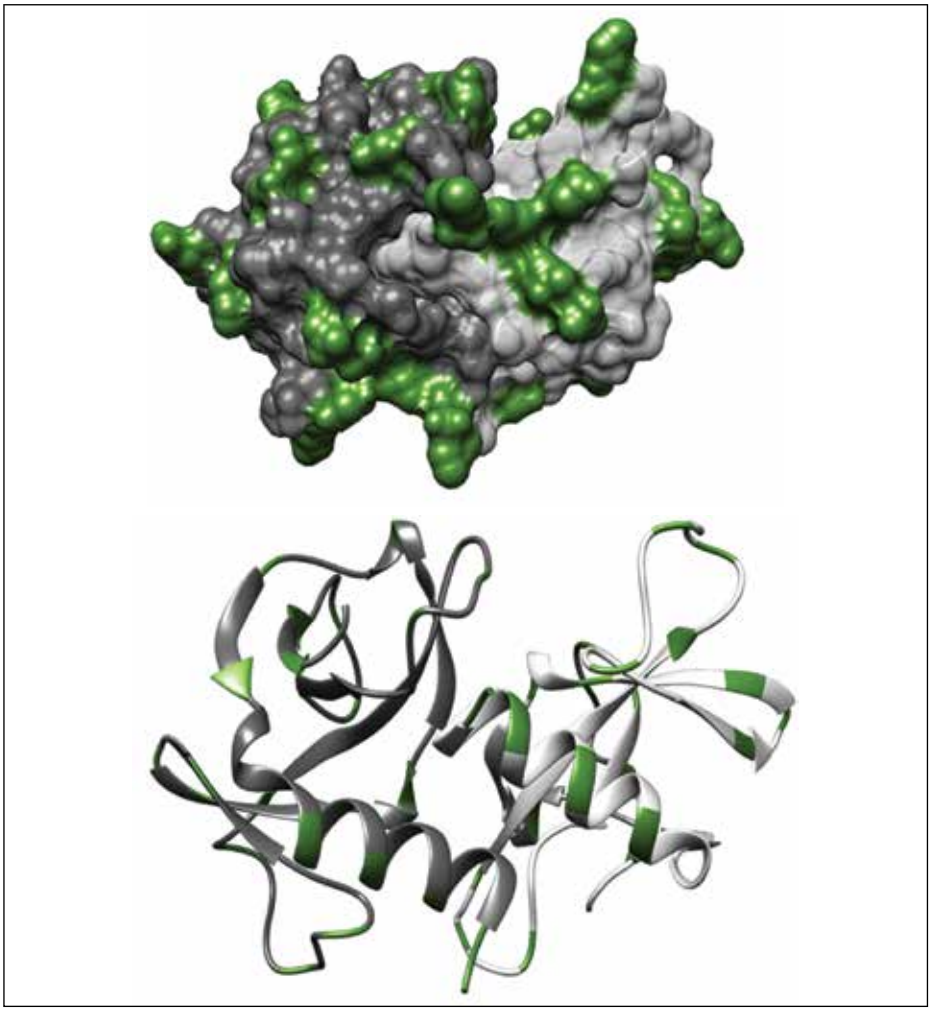
REPRODUCTION AND DEVELOPMENT



Design of Single Cysteine Mutants and Conformational Epitope Mapping

Surface and ribbon diagram of antigen (CcdB protein); light and dark gray colors show the two monomers of CcdB protein, and the green highlighted regions show the positions of residues that were mutated to cysteine for epitope mapping.

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

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One of the themes of research is to study differential gene regulation in Sertoli cells (Sc) during infancy and puberty to identify factors regulating spermatogenesis. We had also undertaken germ cell transplantation studies to restore fertility which is lost as a result of chemotherapy. In addition, we also participate in other endocrinological research as collaborators.

1. To undertake gene expression studies of rat, mice and monkey Sertoli cells to identify factors (genes/miRNA) important for induction of spermatogonial stem cell division and differentiation in the testis.
2. To study biology of spermatogonial stem cells and to use germ cell transplantation technique for restoration of fertility following chemotherapy.
3. To study paracrine and endocrine modulation of signal transduction in target cells of the endocrine system.

Multiomics studies

From our multi-omics approach (TRANSFAC and SWATH-MS), we selected four transcriptions factors namely YY1 and ROR alpha for in-vivo studies. The animals with pubertal Sc specific knockdown of YY1 and ROR alpha were found to have compromised spermatogenesis. RNA-sequencing analysis of YY1 knockdown rats were also performed which suggested its crucial role in gene regulation associated with the functional status of pubertal Sertoli cells.

micro RNA studies

Differentially expressed genes and miRNAs between infant and pubertal Sc were identified. Further investigations revealed a crucial role of microRNA mir-504-5p and Nor-1 in regulation of spermatogenesis. Several differentially expressed genes and miRNAs between infant and pubertal Sc were identified. We undertook gain and loss of function studies

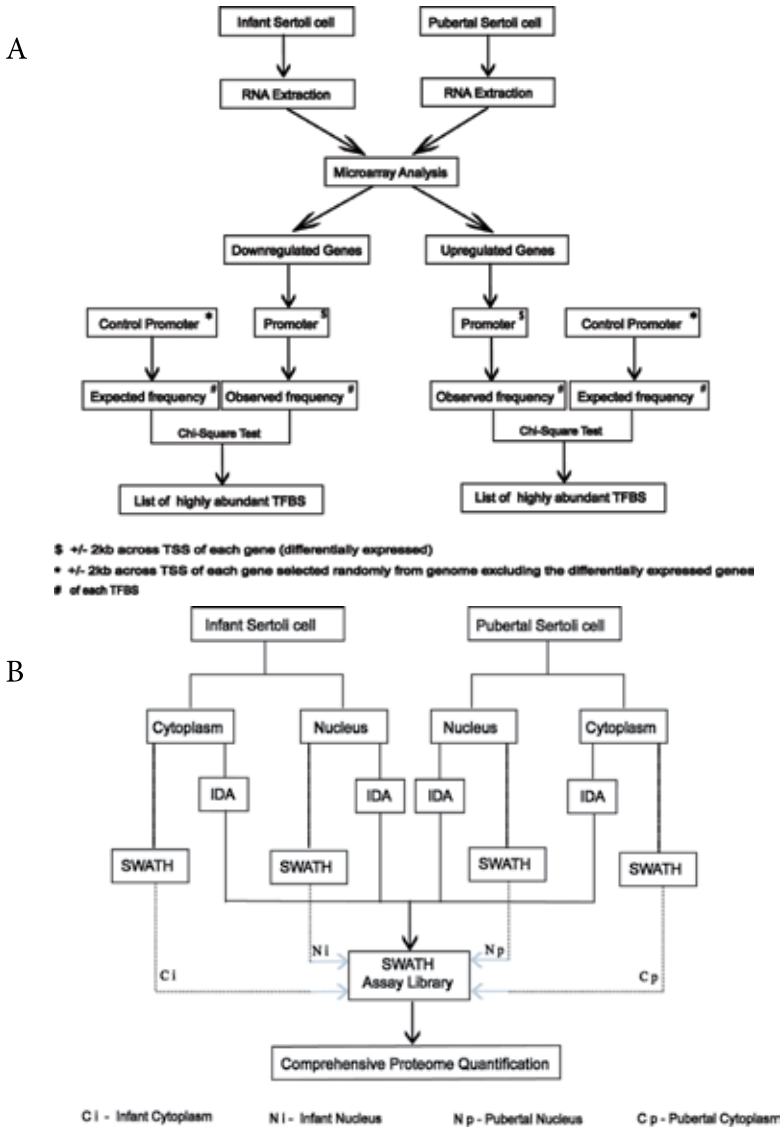

Figure: Workflow used for **(A)** TFBS analysis using TRANSFAC database. **(B)** Comprehensive proteome quantification using SWATH analysis.

in transgenic mouse to further elucidate the role of mir-504-5p in Sertoli cells mediated spermatogenesis. MicroRNA, mir-504-5p is abundantly expressed by mature Sc as compared to infant SCs and we found its crucial role in spermatogenesis. We have generated a transgenic mouse which overexpressed sponge transcript for sequestering (absorbing) mir-504-5p in Sc. Such mir-504-5p functional knockdown in pubertal Sc led to withdrawal of repression on its target, FZD7 leading to augmentation of FZD7 expression. Sponge transgenic mouse displayed sustained expression of Anti-mullerian hormone (AMH) indicating perturbed maturation of Sc. Mir-504-5p knockdown in mouse displayed a significantly attenuated sperm count and litter size. These results revealed for the first time a crucial role of mir-504-5p in Sc maturity and spermatogenesis during puberty.

Role of Hippo Signaling in Sertoli cell maturation

We have identified that the Hippo transducer YAP is an important regulator of cyclic

AMP signaling in functionally mature Sc. Treatment of 19d rat Sc with the YAP inhibitor Verteporfin was found to result in a decline in the expression of cyclic AMP responsive genes like Kitlg, Inhbb, Gja1 which are crucial for spermatogenesis. Verteporfin was also found to attenuate forskolin (adenylate cyclase activator) mediated up regulation of these genes. Further investigations revealed that YAP inhibition induces a sustained phosphorylation of CREB (Cyclic AMP binding protein) in a PKA depend manner. Our results demonstrate, for the first time, a role of this transcriptional co-activator in regulation of cyclic AMP signaling and hence, male fertility.

Endocrine research

A small molecule, a peroxyvanadate compound i.e. DmpzH[VO(O_2)$_2$(dmpz)], referred as dmp was made by Prof Mihir K. Chowdhury of Tezpur University and used in a project to find its ability to control blood sugar levels.This has used animal models of diabetes also.

Publications

Original peer-reviewed articles

1. Mandal K, Bader SL, Kumar P, Malakar D, Campbell DS, Pradhan BS, Sarkar RK, Wadhwa N, Sensharma S, Jain V, Moritz RL, Majumdar SS (2017) An integrated transcriptomics-guided genome-wide promoter analysis and next-generation proteomics approach to mine factor(s) regulating cellular differentiation. *DNA Res* **24** : 147-157.
2. Mishra A, Iyer S, Kesarwani A, Baligar P, Arya SP, Arindkar S, Kumar MJ, Upadhyay P, Majumdar SS, Nagarajan P (2016) Role of antigen presenting cell invariant chain in the development of hepatic steatosis in mouse model. *Exp Cell Res.* **346** : 188-197.
3. Bhattacharya I, Gautam M, Sarkar H, Shukla M, Majumdar SS (2016) Advantages of pulsatile hormone treatment for assessing hormone-induced gene expression by cultured rat Sertoli cells. *Cell Tissue Res.* **368** : 389-396.

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Cell death regulation

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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, our research programme explores the underlying mechanisms of cell survival and death in diverse intracellular and extracellular conditions. The model systems used by us are a lower eukaryotic cell, the protozoan parasite *Leishmania* and the higher eukaryotic mammalian carcinoma cells. Study of both the cellular models is expected to help us understand how complex eukaryotic regulatory systems evolved because some of the cellular pathways may be universal features of eukaryotic cells.

A. Cell death in protozoan parasites

In our efforts to understand the role of host molecules in sustaining *Leishmania* infection, we described an increase in the levels of anti-apoptotic members and a decrease of proapoptotic proteins of the Bcl-2 family post infection. Bcl-2 inhibition either with functional Bcl-2 small molecule inibitors or Bcl-2 siRNA resulted in reduced parasite burden along with an increase in iNOS levels and nitrites as well. These observations clearly indicated a negative effect of Bcl-2 on NO generation. TLR-2 played an important role in Bcl-2 increase, and its downstream effector appeared to be the ERK pathway. IL-13 was linked to Bcl-2 elevation post infection. Leads from the *in vitro* and *in vivo* mouse studies suggested a possible functional role of IL-13 in VL. VL patients showed high IL-13 and low nitrites in their sera, with IL-13 levels dropping after treatment. Continuing our studies with the two defensive enzymes, the cytosolic and the mitochondrial tryparedoxin peroxidases, namely cTXNPx and mTXNPx, we report that parasites expressing inactive enzymes are not able to create successful infections.

B. Mechanisms underlying cell death in cancer

Sestrins are stress inducible antioxidant proteins that regulate many events in the cell. Our studies provide important clues on how sestrins could

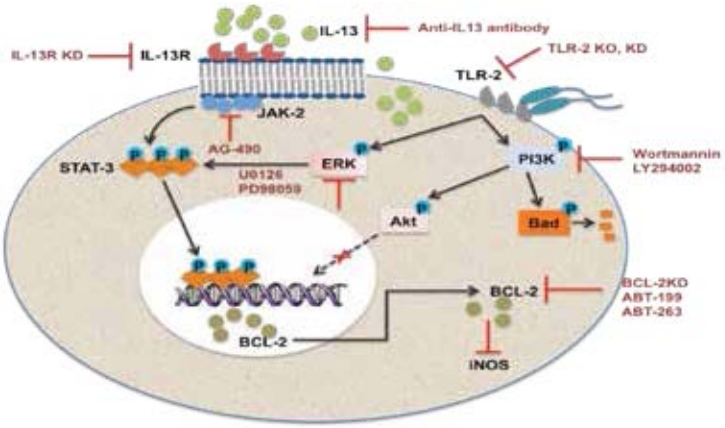

Figure:1 A schematic diagram showing the regulation of Bcl-2 and NO during infection with the *Leishmania* parasite. Inhibitor names are given in brown color with inhibitor bars in red.

be regulating mitophagy, as it is an essential event for cell survival. We show that SESN2 downregulation retards Beclin-1 phosphorylation at ser14 position by preventing phosphorylation of ULK-1 kinase at position ser555. These events lead to abrogation of parkin translocation during CCCP treatment through lowering of Beclin-1 and parkin interaction. The importance of the event is indicated by increased cell death when SESN2 is lowered.

The future plans will be to complete our programme on the defense mechanisms in the protozoan parasite *Leishmania* in relation to cell death. Further studies on the mechanisms by which the *Leishmania* parasite survives within neutrophils is being pursued as neutrophils are temporary but important hosts in the early part of infection.

Publications

Original peer-reviewed articles

1. Pandey RK, Mehrotra S, Sharma S, Gudde RS, Sundar S, Shaha C (2016) *Leishmania donovani*-Induced Increase in Macrophage Bcl-2 Favors Parasite Survival. *Front Immunol.***7**:456.
2. Singh AK, Pandey RK, Shaha C and Madhubala R (2016) MicroRNA expression profiling of *Leishmania donovani* infected host cells uncovers the regulatory role of MIR-30A-30 in host autophagy. *Autophagy* **12**: 1817- 1831.

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2. Mathur R, Shaha C (2016) Cell death profile of a protozoan parasite. In ***Recent Advances in Communicable and Non-communicable diseases***. (Eds. A. Dutta and V.P. Sharma, NASI- Capital Publishing. New Delhi) pp 244-255.

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Cellular and molecular aspects of reproduction and herbal formulations for prevention of sexually transmitted HIV-1

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Development of contraceptive vaccine

Immunization of female FVB/J mice with *E. coli*-expressed recombinant $Sp17_C$-$GnRH_2$ and $GnRH_2$-$Sp17_C$ led to higher antibody titres against $Sp17_C$ and GnRH in $Sp17_C$-$GnRH_2$ immunized group. Further, 90% of the female mice immunized with recombinant $Sp17_C$-$GnRH_2$ failed to conceive as compared to 30% females immunized with $GnRH_2$-$Sp17_C$. These studies suggested that the recombinant $Sp17_C$-$GnRH_2$ is able to appropriately display $Sp17_C$ as well as GnRH to have optimum immune response and contraceptive efficacy.

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

i) Trophoblastic cell migration

Silencing of Wnt4, Wnt11, Intα2 & IntαV respectively by siRNA led to significant decrease in the HGF–mediated migration of HTR-8/SVneo cells. Further studies established a cross-talk between Wnts and integrins during HGF-mediated migration of trophoblastic cells.

ii) Trophoblastic cell invasion

***Effect of cytokines and growth factors*:**

Using siRNA, Basic Leucine Zipper ATF-Like Transcription Factor 2 (BATF2) was shown to play an important role in IFNγ-mediated decreases in invasion of the HTR-8/SVneo cells. Phosphorylation of STAT1 at ser 727 and tyr 701 residues was important during decrease in invasion after treatment with IFNγ. Cross-talk between STAT1 and BATF2 was also established.

During EGF-mediated increase in invasion of HTR-8/SVneo cells, phosphorylation of ERK½, STAT1 and STAT3 was observed. Silencing

of STAT1 led to significant increase in the basal invasiveness of HTR-8/SVneo cells but no significant increase in the invasiveness on treatment with EGF was observed (Fig. 1). STAT1 silencing also led to decrease in phosphorylated ERK½ and P-STAT3 ser 727 (Fig. 1). After EGF treatment, activation of MAPK and JAK-STAT pathways are interdependent on each other.

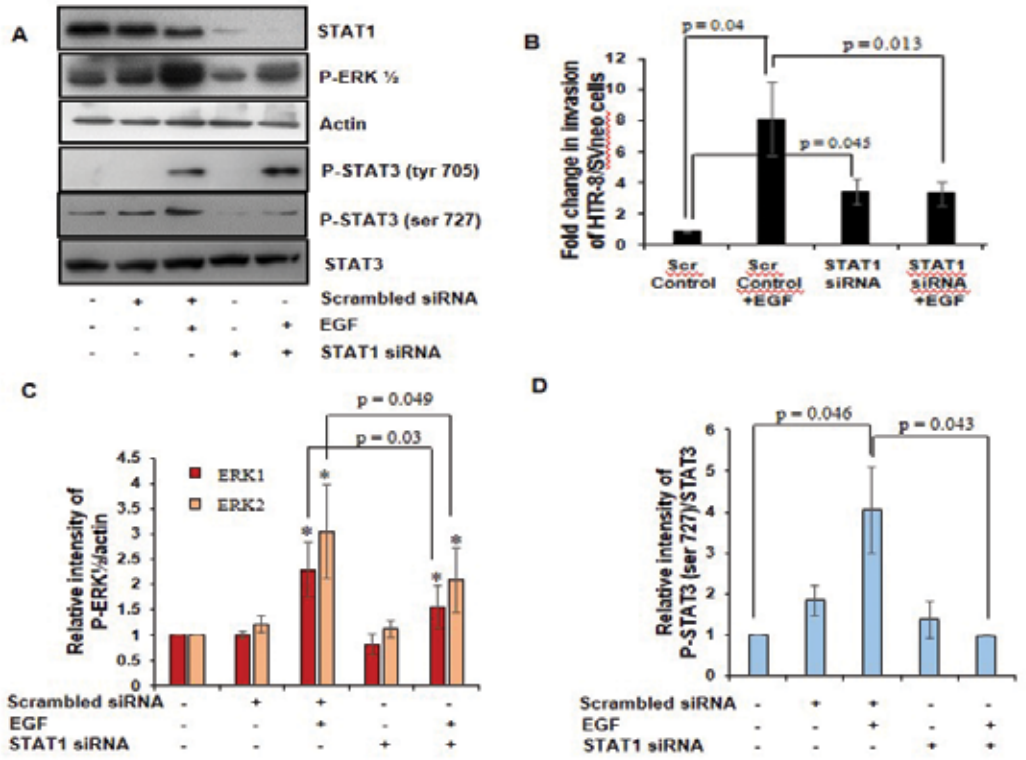

Figure 1: **Effect of STAT1 silencing on the invasion and downstream signaling of HTR-8/SVneo cells in presence or absence of EGF.** STAT1 was silenced in HTR-8/SVneo cells using siRNA. The naïve cells, scrambled siRNA and STAT1 siRNA transfected cells were treated with EGF (10 ng/ml). Panel A show representative blots from one of the three independent Western blot experiments. Panel B shows relative fold change in invasion following EGF treatment of scrambled siRNA and STAT1 siRNA transfected cells as compared to HTR-8/SVneo cells transfected with scrambled siRNA without EGF treatment. Panels C and D represent densitometric profiles of phosphorylated ERK½ as compared to actin, phosphorylated STAT3 at ser727 as compared to total STAT3 respectively in naïve cells, scrambled and STAT1 siRNA treated cells in presence or absence of EGF.

***Effect of oxidative stress*:** To understand role of reactive oxygen species during trophoblast invasion, treatment of HTR-8/SVneo cells with 25 µM of H_2O_2 led to a significant increase in the expression of IL-8 and MIP-1β. In addition, activation of STAT-1 (ser 727) and STAT-3 (ser 727) as well as significant increase in MMP-9/TIMP-1 ratio was also observed.

iii) Trophoblastic cell differentiation

Increase in BeWo cell differentiation on treatment with forskolin/hCG involves increased expression of Wnt10b, which in turn is regulated by activation of PKA. Inhibition of PKA by H89 also showed a lower nuclear/cytoplasmic active β-catenin expression along with reduced syncytin-1 expression. These and previous studies led us to conclude that Wnt10b has a role in forskolin-/hCG-mediated trophoblastic BeWo cell fusion via β-catenin/GCMa/syncytin pathway (Fig. 2).

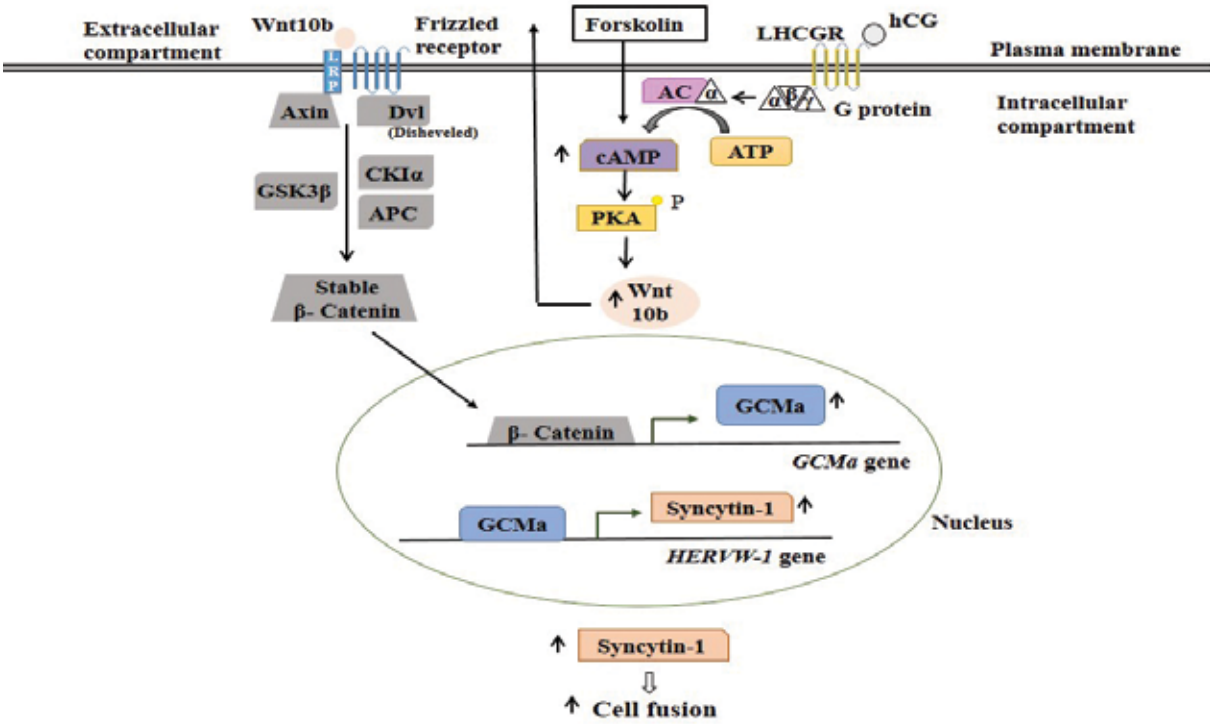

Figure 2: Mechanistic overview of the role of Wnt10b in BeWo cell fusion. Increase in forskolin/hCG-induced Wnt10b expression may fall downstream of cAMP/PKA signaling. Increased expression of Wnt10b leads to stabilization of β-catenin expression via canonical pathway, which in turn leads to an increase in GCMa as well as syncytin-1 expression. Therefore, Wnt10b lead to increased BeWo cell fusion via β-catenin/GCMa/syncytin pathway.

Gel based herbal formulations for prevention of HIV-1 infection

Both 4 and 5 plants-based herbal gel formulations showed dose dependent inhibition of HIV-1 infection in human PBL. Formulations were stable when stored up to 3 months at 40°C. The herbal formulations did not show any significant increase in the mutagenic index using *Salmonella typhimurium* strains TA-98 and TA100. An Indian patent on these formulations for anti-HIV-1 activity has been jointly filed with HLL Lifecare India Ltd, Thiruvananthapuram.

Publications

Original peer reviewed articles

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2. Debnath U, Kumar P, Agarwal A, Kesharwani A, Gupta SK, Katti SB (2017) N-hydroxy substituted 2-aryl acetamide analogues: a novel class of HIV-1 integrase inhibitors. *Chem Biol Drug Des.* DOI: 10.1111/cdbb.12974 .
3. Najar TA, Khare S, Pandey R, Gupta SK, Varadarajan R (2017) Mapping protein binding sites and conformational epitopes using cysteine labeling and yeast-surface display. *Structure* **25**: 395-406.
4. Kesharwani A, Polachira SK, Nair R, Agarwal A, Mishra NN, Gupta SK (2017) Anti-HSV-2 activity of *Terminalia chebula* Retz extract and its constituents, chebulagic and chebulinic acids. *BMC Complement Altern Med.* **17**:110.
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Patent

1. Polachira SK, Nair R, Jayalekha R, Gupta SK, Mishra NN and Agarwal A (2017) Herbal microbicide formulation for preventing HIV (*Indian patent application no.* 201741004017 *filed on 3/02/2017*).

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Modulation of intracellular trafficking in host cells by various intracellular pathogens

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Metalloprotease gp63 (Ldgp63) is a critical virulence factor secreted by *Leishmania*. However, how newly synthesized Ldgp63 exits the endoplasmic reticulum (ER) and is secreted by this parasite is unknown. We have characterized the COPII components like LdSar1, LdSec23, LdSec24, LdSec13, and LdSec31 from *Leishmania* to understand their role in ER exit of Ldgp63. Using dominant-positive (LdSar1:H74L) and dominant-negative (LdSar1:T34N) mutants of LdSar1, we have found that GTP-bound LdSar1 specifically binds to LdSec23, which binds, in turn, with LdSec24$^{1-702}$ to form a prebudding complex. Moreover, LdSec13 specifically interacts with His$_6$-LdSec31$^{1-603}$, and LdSec31 binds with prebudding complex via LdSec23. Interestingly, dileucine$^{594/595}$ and valine^{597} residues present in the Ldgp63 C-terminal domain are critical for binding with LdSec24$^{703-966}$, and GFP-Ldgp63$^{L594A/L595A}$ or GFP-Ldgp63^{V597S} mutants fail to exit from the ER. To directly demonstrate the function of LdSar1 in Ldgp63 trafficking, we have coexpressed RFP-Ldgp63 along with LdSar1:WT-GFP or LdSar1:T34N-GFP and have shown that LdSar1:T34N overexpression blocks Ldgp63 trafficking and secretion in *Leishmania*. Finally, we have found that LdSar1:T34N-GFP overexpressing transgenic parasites survival is significantly compromised in macrophages. Therefore, the development of appropriate inhibitors or small molecules targeted to parasite COPII proteins could be new therapeutic approach against the parasites.

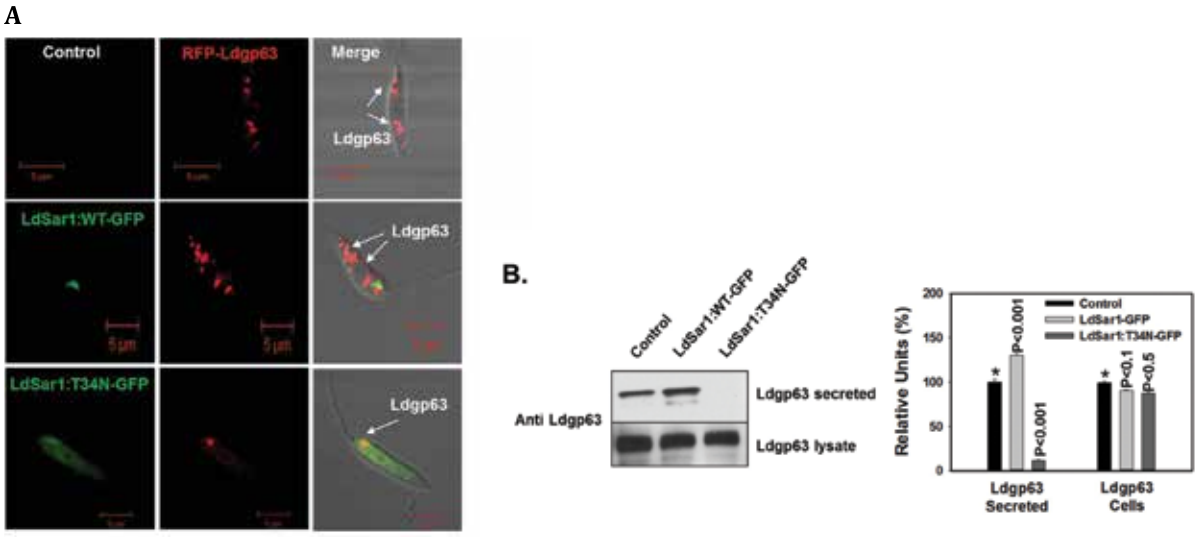

Figure. 1. Dominant negative mutant LdSar1:T34N blocks the trafficking and secretion of Ldgp63 in *Leishmania*.

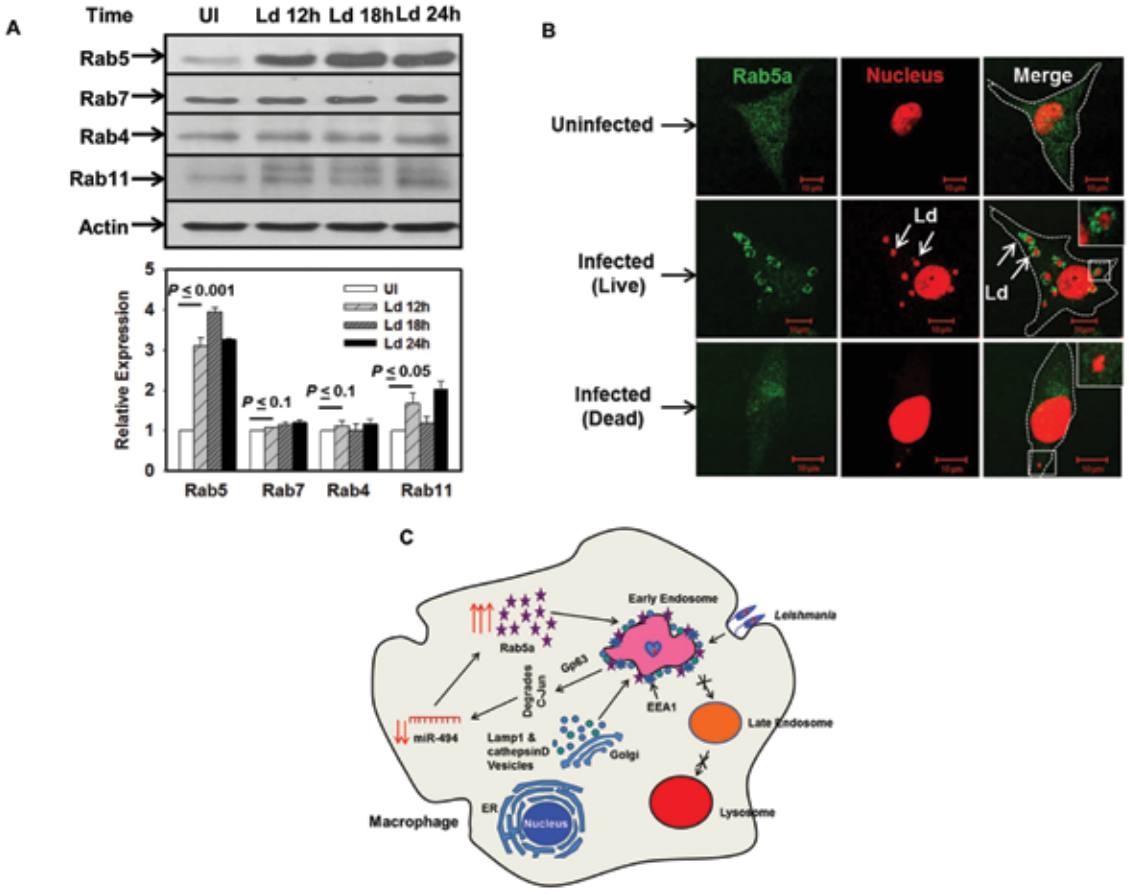

Figure. 2. Leishmania over-expresses Rab5a in host macrophages and recruits Rab5a on PV by degrading host miR-494.

Several intracellular pathogens arrest the phagosome maturation in the host cells to avoid transport to lysosomes. In contrast, the *Leishmania* containing parasitophorous vacuole (PV) is shown to recruit lysosomal markers and thus *Leishmania* is postulated to be residing in the phagolysosomes in macrophages. In another study, we have shown that *Leishmania donovani* specifically upregulates the expression of Rab5a by degrading c-Jun via their metalloprotease gp63 to downregulate the expression of miR-494 in THP-1 differentiated human macrophages. Our results have also shown that miR-494 negatively regulates the expression of Rab5a in cells. Subsequently, *L. donovani* recruits and retains Rab5a and EEA1 on PV to reside in early endosomes and inhibits transport to lysosomes in human macrophages. However, the parasite modulates the endosome by recruiting Lamp1 and inactive pro-CathepsinD on PV via the overexpression of Rab5a in infected cells. Furthermore, siRNA knockdown of Rab5a or overexpression of miR-494 in human macrophages significantly inhibits the survival of the parasites. These results provide the first mechanistic insights how *Leishmania* modulates endo-lysosomal trafficking in macrophages to reside in a specialized early endocytic compartment.

Publications

Original peer reviewed articles

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2. Shanmugam SK, Kumar K, Singh PK, Rastogi R, Mukhopadhyay A (2016) Single GDP-dissociation Inhibitor Protein regulates endocytic and secretory pathways in *Leishmania*. *Sci Re*p. **6**:37058.
3. Mukhopadhyay A, Sehgal L, Bose A, Gulvady A, Senapati P, Thorat R, Basu S, Bhatt K, Hosing AS, Balyan R, Borde L, Kundu TK, Dalal SN (2016) 14-3-3γ prevents centrosome amplification and neoplastic progression. *Sci Rep*. **6**:26580.

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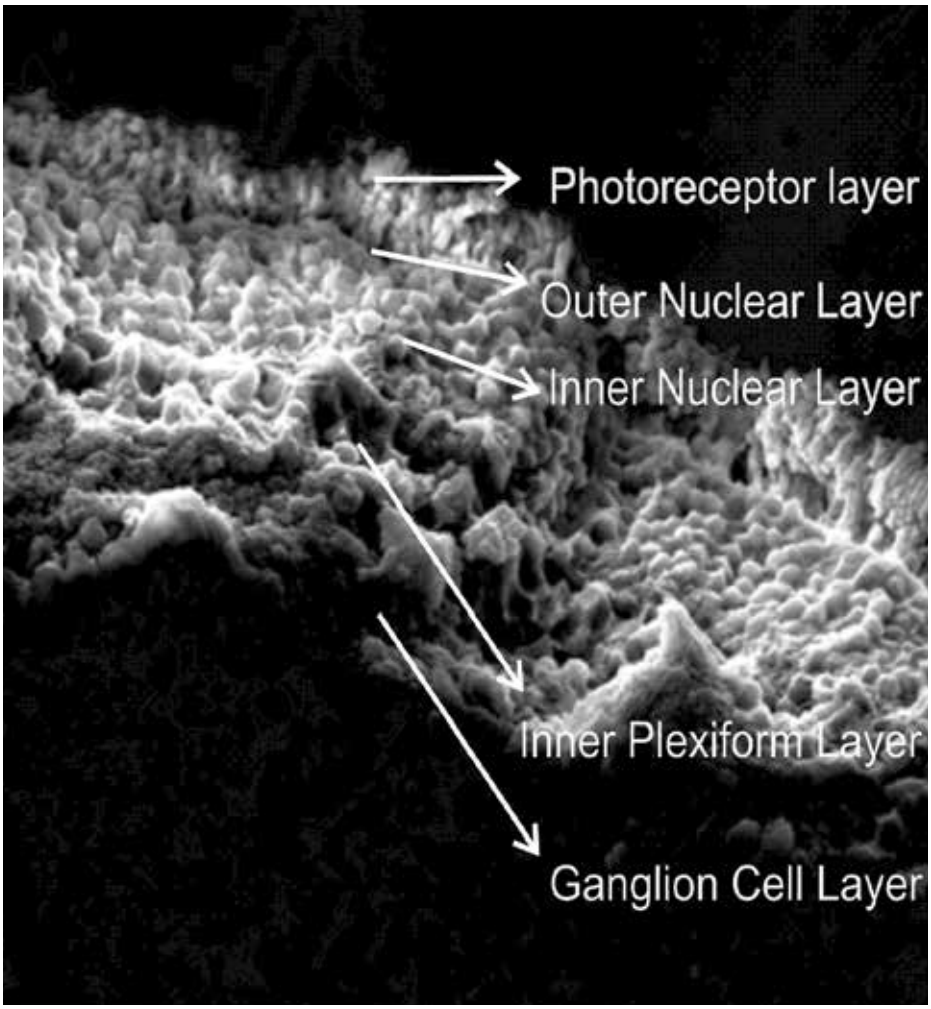
MOLECULAR DESIGN



Scanning Electron Microscopic image showing topology and structure of a retina.

Structural studies on proteins, dynamics and ligand interactions using NMR

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The theme of our research is to understand the structure, dynamics, and ligand interactions of proteins using NMR. Presently, we are working on some of the key proteins of the fatty acid metabolism of *Leishmania viz*. the acyl carrier protein, enzymes of type II fatty acid biosynthesis pathway, as well as the proteins involved in the dispersal of acyl CoAs.

Structural characterization of the proteins involved in fatty acid dispersal in *Leishmania*

Fatty acids are important biomolecules, necessary for the survival of the amastigote stage of *Leishmania*. Despite its ability to absorb lipids directly from the host through parasitophorous membrane, fatty acids/ cholesterol present in the host cells need remodelling, as their composition is different from that required by *Leishmania*. Hence, they are esterified to coenzyme A derivatives first, prior to their oxidative degradation and utilization for remodelling. The acyl-CoAs thus generated are transported to the site of elongase function for further extension, by specialized carrier proteins, called the acyl-CoA binding proteins.

Based on genome sequence, there are three free standing acyl-CoA binding proteins in *Leishmania*; $ACBP_{89}$, $ACBP_{96}$ and $ACBP_{103}$. Isothermal titration calorimetry studies were carried out to determine the binding affinity of these three proteins towards various acyl-CoAs. $ACBP_{89}$ displayed strongest binding to C_{14}-CoA, while $ACBP_{103}$ towards C_{18}-CoA, as shown in Figure 1.

In order to understand the the molecular basis of ligand specificity, and in turn function of *Lmj*$ACBP_{103}$, we have solved its crystal structure at 1.5Å resolution.

The crystal structure of *Lmj*$ACBP_{103}$ is comparable to other ACBP structures, comprising of four helices, enclosing a large cavity present at the bottom, that binds

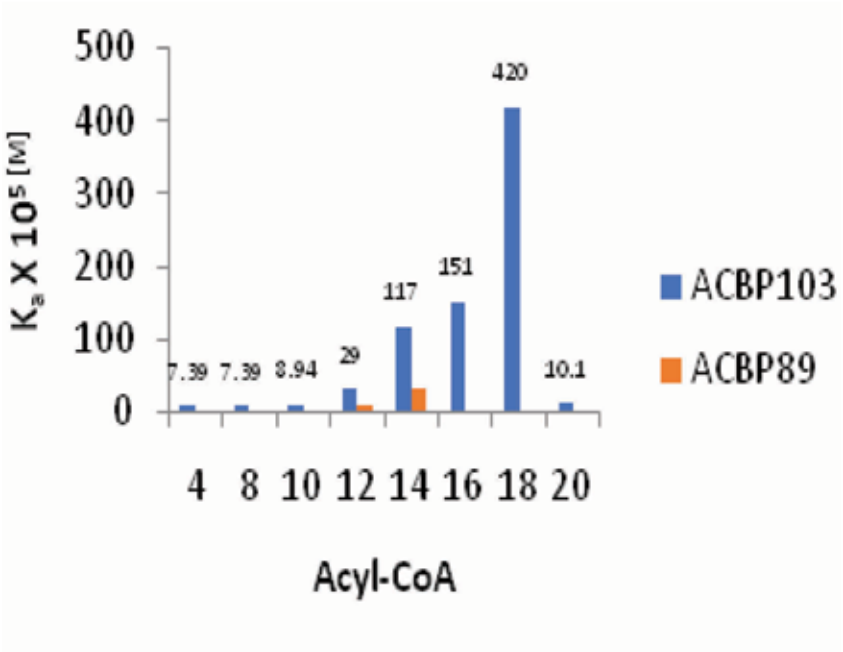

Figure. 1. Binding affinity of *Lmj*ACBP$_{89}$ and *Lmj*ACBP$_{103}$ towards various acyl-CoA, K_a determined using ITC.

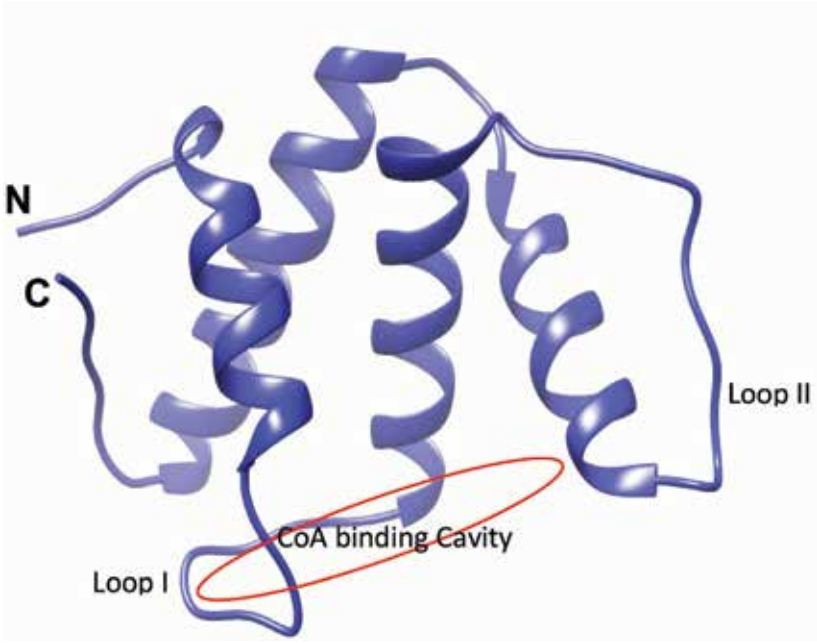

Figure. 2. A ribbon representation of the structure of LmjACBP$_{103.}$

the ligand as illustrated in Fig. 2. Loop I of *Lmj*ACBP$_{103}$ has four extra residues, compared to other ACBPs. Interestingly, the residues of ACBP known to bind CoA, and the phosphate are fully conserved. Apparently, the 4 gatekeeper residues at the end of the ligand binding tunnel are remarkably different.

NMR studies were also carried out to gain insights into the mechanism of interaction of *Lmj*ACBP$_{103}$ with its ligand. Major chemical shift changes were observed in the C-terminus of helix A_1, loop I, and N-terminus of helix A_2, as shown in Fig. 3. The ligand fits in a lipophilic tunnel, present between helix A_2 and A_3. The end of the tunnel is lined by Asn 26, Leu 30, Val 54, and Ala 58 based on our NMR studies.

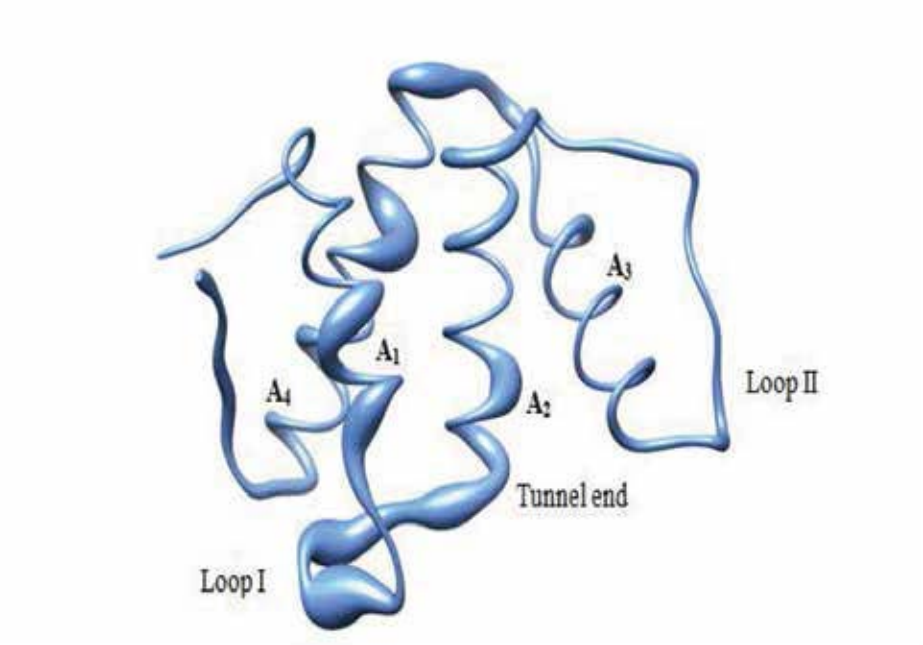

Figure. 3. A worm representation of ACBP$_{103}$, highlighting the residues that display chemical shift perturbations.

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To develop strategies for making sensors and actuators for biological processes

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A. Hepatocytes like cells from PBMCs

The NeoHep differentiated from human blood monocytes were transplanted in a partially hepatectomised NOD.SCID mouse and a few important hepatocyte specific functional features of NeoHeps were investigated.

Secretion of Active Clotting Factor VII

Clotting factor VII is a vitamin K dependent plasma glyco-protein and apart from albumin secretion, it is one of the major clinical functions performed by hepatocytes. Figure 1, Panel A, confirms that both healthy and HNP NeoHep were able to secrete functionally active clotting factor VII in culture supernatant in contrast to reprogrammed monocytes (RM), from both the sources.

Presence of Glycogen

Synthesis and storage of glycogen are the important functions of hepatocytes. The Panel B of Figure 1 shows the PAS staining in NeoHep unlike monocytes and RM. Haematoxylin staining was performed for the identification of nucleus.

(A) Clotting Factor VII Activity in Culture Supernatant. Fig. showing the clotting factor VII activity in cell culture supernatant of RM and NeoHep of both Healthy and HNP samples supplemented with vitamin K. (N=3) *=$p<0.05$

(B) Presence of Glycogen. Representative bright field images of PAS staining for the detection of Glycogen along with hematoxylin in Monocyte, RM and NeoHep of healthy samples under 20X objective.

(C) Storage of Lipid. Representative bright field images of Oil red O staining for the detection of lipid droplets along with hematoxylin in Monocyte, RM and NeoHep of healthy sample under 20X objectives.

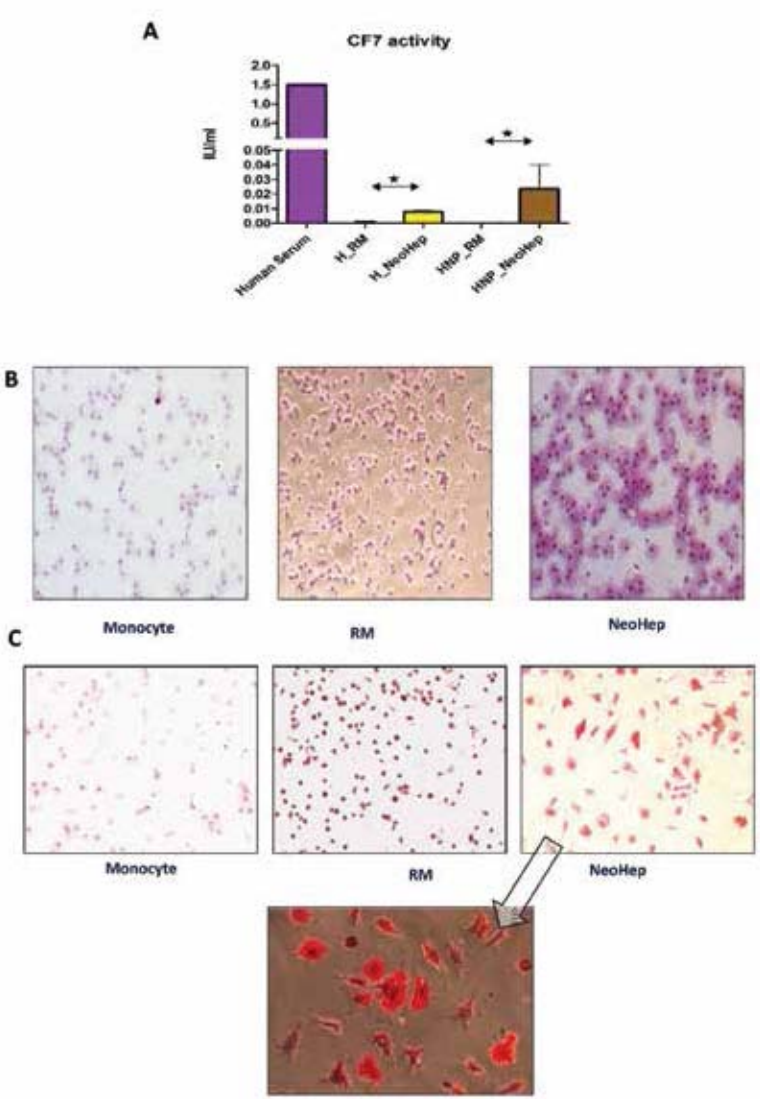

Figure 1. Functional Characterization of NeoHep: Clotting Factor VII activity in culture supernatant in presence of vitamin K: Fig. showing the clotting factor VII activity in cell culture supernatant of RM & NeoHeps of both Healthy & HNP samples supplemented with vitamin K. The bar represents the mean value of clotting factor VII activity in terms of IU/ml. Human serum was taken as positive control for this assay. Error bar signifies the standard error of mean. (N=3) *=p<0.05. Presence of glycogen: Representative bright field images of PAS staining for the detection of Glycogen along with hematoxylin in Monocyte, RM and NeoHeps of healthy samples under 20X objective. Storage of lipid: Representative bright field images of Oil red O staining for the detection of lipid droplets along with hematoxylin in Monocyte, RM and NeoHep of healthy sample under 20X objectives. The images in zoom signifies the presence of lipid droplets in the forms of globules in one of representative NeoHep samples.

B. Development of acute liver failure (ALF) model in rats

By combining hepatectomy and hepatotoxic chemicals we are developing an animal model of ALF in lesser time with lower doses of hepatotoxic reagents. ALF condition, confirmed by relevant gene expressions and serum levels of enzymes secreted by liver, was generated by combining 70 % hepatectomy and acetaminophen (750 mg/kg) drug administration and death was observed in operated rats within 48 hrs of acetaminophen dose administration.

C. Integration studies of retinal neuron like cells (RNLCs) in NOD.SCID-*rd1* mice

RNLCs were transplanted subretinally in the eye of NOD.SCID-*rd1* animals (3×10^6 cells)

and eyes were isolated post 48 hours after euthanizing the animals. Around 1.25 % of RNLCs were integrated in the degenerated retina upon transplantation. The serum levels of c-GMP was significantly reduced in post transplanted animals along with slightly down regulated levels in the eye lysate samples of post transplanted animals.

We found that there was significant number of cells stained for retinal markers rhodopsin and PKC-a, present in INL layer of retina and CRALBP in the INL and GCL layers suggesting the viability and functionality of the transplanted cells.

The ERG analysis post transplantation revealed that both a and b waves displayed improvement as compared to non-transplanted eye. There was around 15-20 % rescue in the a wave and b wave also indicated marked improvement at day 3 post transplantation. The improvement further enhanced to 25-30 % in a wave at 10 days post transplantation while b wave did not show much change at day 10.

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Original peer-reviewed articles

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3. Mishra A, Das B, Nath M, Iyer S, Kesarwani A, Bhattacharjee J, Arindkar S, Sahay P, Jain K, Sahu P, Sinha P, Velpandian T, Nagarajan P, Upadhyay P (2017) Novel immunodeficient Pde6b rd1 mouse model of retinitis pigmentosa to investigate potential therapeutics and pathogenesis of retinal degeneration. *Biol Open* **6**:449-462

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Ankita Dabla
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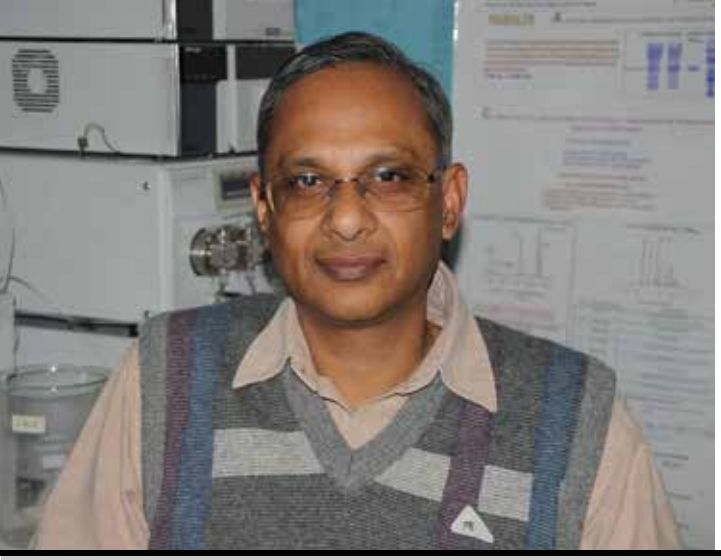

Protease-catalyzed splicing of peptide bond

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We are interested in peptide ligation reactions catalyzed by proteases/ transpeptidases with a view to develop chemo-enzymatic strategies, for engineering non-standard chemical functionalities in proteins, and construction of well defined bioconjugates. Our current studies are focused on a unique class of transpeptidase (referred to as sortase) found in gram-positive bacteria. Sortase performs the covalent anchoring of surface proteins *via* a peptide ligation reaction in which LPXTG- containing polypeptides are linked to the terminal Gly residue of the pentaglycine arm of the peptidoglycan. Sortase has emerged as a useful tool in synthetic protein chemistry. Newer sortase enzymes with enhanced efficiency, stability and diverse substrate specificity are desired for expanded applications.

Studies on structure, dynamics and function of sortases.

Studies on substrate specificity of a new class of housekeeping sortase namely, SavSrtE of *Streptomyces avermitilis* was reported. The ''LPXTG" peptide substrate which is recognized by the classical A class sortase (SaSrtA) of *Staphylococcus aureus* was found to be a relatively poor substrate for SavSrtE. This class E enzyme preferred a "LAXTG" motif juxtaposed with non-polar residues and displayed strict specificity for aminoglycine-based nucleophilic substrates.

The major focus of the continued work was to glean insights as to why LPXTG peptide was not preferred by SavSrtE. Based on the crystal structure and modeling studies, we probed the possible role of Y112 residue (equivalent to Ala104 in SaSrtA). We generated four mutants of SavSrtE by replacing Tyr112 by Ala, Gly, Trp and Phe. The ability of mutants to catalyze transpeptidation reaction was explored with YNLAETGA (Ala-substrate) and YNLPETGA (Pro-substrate) using GGGKY as an acceptor peptide. The assay revealed abrogation of

activity in Ala, Gly and Trp mutants. However, Y112F mutant was active on both substrates *albeit* to different extent. The Y112F mutation did not alter the Pro-specificity but caused about 50-fold decrease in K_{cat}/K_m for the Ala-substrate. Analyses of steady state parameters of Y112F mutant showed a 15-fold shift in the ratio of Ala/Pro specificity from SaSrtA. The corresponding shift for the wild type SavSrtE was found to be 600-fold. The kinetic assays therefore, clearly demonstrated overall alteration in substrate specificity of SavSrtE and established an important role for Y112 residue in dictating the Ala-specificity. These results are instructive in view of the fact that no single residue of a sortase in isolation has been seen to exert significant discriminatory influence on substrate preference.

Sortase-mediated protein labeling and conjugation.

We reported a sortase-mediated strategy for synthesis of SUMO-conjugates that can serve as substrates for desumoylating enzymes. In the past year, we elaborated the semisynthesis of SUMO-conjugates comprising isopeptide-linked target peptide sequences derived from RanGAP1 and C-Jun to establish the generality of the approach for generating *bona fide* substrates for deconjugating enzymes (SENPs).

We have also initiated studies on semi synthesis of histones with an aim to obtain a homogeneous preparation of precisely modified histone. The post translational modifications in histones are largely located in the unstructured N-and C-terminal regions and are accessible to sortase-mediated ligation strategy. Currently, we are in the process of engineering a mutation (Ala30-Leu) in H2A to generate a sortase-recognition motif.

Publications

Original peer-reviewed articles

1. Das S, Pawale VS, Dadireddy V, Singh AK, Ramakumar S, Roy RP (2017) Structure and specificity of a new class of Ca^{2+} independent housekeeping sortase from *Streptomyces avermitilis* provides insights into its non-canonical substrate preference. *J Biol Chem* **292**: 7244–7257.
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Therapeutic interventions in chronic diseases

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My group is a multi-disciplinary group adapting an integrated approach in drug discovery that combines medicinal chemistry, basic biology and biochemistry principles for efficient drug design process. 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.

To study the effect of testosterone supplementation on hepatic insulin responsiveness and glucose homeostasis through liver in male high fat diet (HFD) induced Type 2 Diabetes Mellitus (T2DM) mice.

Effect of testosterone on Glucose homeostasis in liver

Liver is one of the major organs involved in glucose homeostasis in the body. During extended fasting, the liver converts pyruvate to glucose, by a process called gluconeogenesis, to maintain normoglycemic level where Phosphoenolpyruvate carboxykinase (PEPCK) being the rate limiting enzyme. Under normal conditions, once the normoglycemia is attained, insulin inhibits further hepatic glucose production by inhibiting gluconeogenesis. However, in Type 2 Diabetes Mellitus (T2DM), the body is not able to effectively utilise insulin to maintain normoglycemic level and the hepatic glucose output is not in the ambit of control of insulin and leads to hyperglycemia, which is reflected by higher fasting blood glucose level (BGL). Clinical studies have revealed that testosterone supplementation had a positive effect on glucose homeostasis in T2DM but did not address how testosterone supplementation affected insulin responsiveness in liver, a key glucose homeostatic organ. In this study, we aimed to study the effect of testosterone supplementation on hepatic insulin responsiveness and glucose homeostasis through liver in male high fat diet (HFD)

induced Type 2 Diabetes Mellitus (T2DM) mice.

The testosterone Treated animals showed lower fasting BGL(a marker of hepatic glucose output) as compared to the Control for over a period of 28 weeks (Fig. 1B). Hepatic glucose output quantified by the Pyruvate Tolerance Test (PTT) showed lesser increase in BGL of Treated animals as compared to Control (Fig. 1C-E). This indicate reduced hepatic glucose output during long hours of fasting and hence better glycemic control upon testosterone administration in T2DM male mice. Interestingly, we observed no significant change in the levels of key glucose homeostatic hormones in the serum of Treated and Control animals (Fig. 2A). This suggests that testosterone supplementation did not affect hepatic gluconeogenesis through these glucose homeostatic hormones. Instead it could have altered signalling in the liver, which led to reduced hepatic glucose output in the testosterone administered T2DM males. We checked the PEPCK level and found significantly lower level of PEPCK in liver of the Treated animals (Fig. 2B). Thus significant decrease in PEPCK might result in reduced hepatic glucose output in the Treated animals, as depicted by fasting BGL and PTT. As FOXO1 is a regulator of PEPCK, we checked FOXO1 level upon testosterone administration. Surprisingly, we found an increased level of total FOXO1 in liver of Treated animals as compared to the Control (Fig. 2C). Increased level of FOXO1 is related to insulin resistance. Insulin resistance leads to decreased P-AKT (Ser-473) levels or causes AKT inactivation and hence the inactivation of FOXO1 is reduced. In increased insulin resistance condition, there is reduction in P-AKT levels upon insulin treatment and increased FOXO1. Thus, we investigated the P-AKT (Ser-473) level, which is also a marker for tissue insulin responsiveness, in the liver of Treated and Control animals.

We observed that the P-AKT levels were highest and FOXO1 levels were least in the Control animals which were also given insulin. The Treated animals upon insulin administration had the highest FOXO1 and signifinantly lower P-AKT (Ser-473) level than the insulin treated Control (Fig. 2D-E). There was no significant difference in the P-AKT (Ser-473) level of Control and Treated animals. The increase in P-AKT (Ser-473) level in Treated animals upon insulin administration was insignificant, which indicates impaired insulin action/signalling in liver of Treated animals, whereas a significant increase in P-AKT (Ser-473) level upon insulin administration in Control animals suggest better insulin response in liver (Fig.2E). We probed for P-FOXO1 (Ser-256) and found significantly higher level of P-FOXO1 (Ser-256) in the insulin administered Control as compared to the insulin administered Treated animals (Fig. 2F).

It has been reported that the interaction between FOXO1 and AR (which is independent of PI3K-AKT) led to proteosamal degradation of FOXO1 to a 60 kDa product and transcriptional activity of FOXO1 was inhibited. We found a profound 60 kDa band in the T+I sample (Fig. 2E), which is possibly due to proteosomal degradation of FOXO1 upon interaction with AR. The interaction between FOXO-1 and AR was further confirmed by immunoprecipitation of Foxo-1 from tissue lysate and probe for AR (Fig 2F). The AR-FOXO1 interaction prevented DNA binding of FOXO1 and had an inhibitory effect on transcriptional activity of FOXO1, which could possibly be the reason for lower PEPCK levels in liver of Treated animals despite increase in insulin resistance. These results were validated in Hepg2 cell line studies. Next we went on to check the level of P- GSK3α (Ser-21) in the liver of Treated and Control animals as GSK3 also plays an important role in PEPCK regulation and glucose homeostasis in liver.

In our experiment, we found increased GSK3α inactivation, i.e. higher levels of P- GSK3α (Ser-21), in the liver of Treated animals. Though our previous experiments depict increased insulin resistance in the liver of Treated animals, the testosterone treatment resulted in increased GSK3α inactivation. As we found maximum inactivation of GSK3α in the presence of only testosterone even when PI3K/AKT pathway was blocked, suggesting testosterone mediated inhibition of GSK3α in liver was independent of PI3K/AKT pathway. This suggest that despite increased hepatic

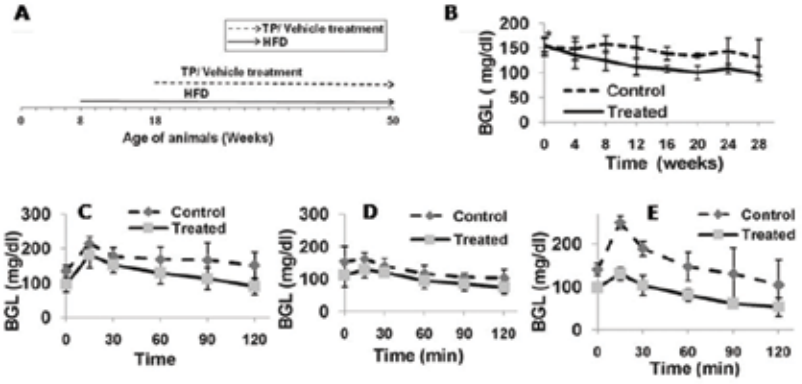

Figure 1. A: Schematic representation of HFD feeding and treatment duration in animals. **B:** Reduced hepatic glucose output in Treated animals. B: Fasting BGL of Control and Treated animals. **C-E:** PTT in Control and Treated animals after 4 (C), 16 (D) and 32 (E) weeks of treatment.

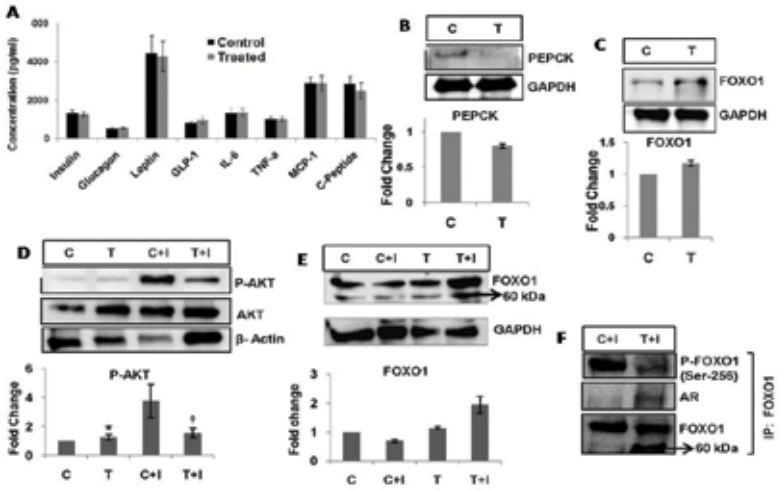

Figure 2. A: No significant change in serum level of key glucose homeostatic hormones and cytokines. Serum levels of analytes involved in glucose homeostasis in Control and Treated animals. n= 6, p-value> 0.1. B-C: Immunoblot analyses depict reduced PEPCK level but increased insulin resistance in liver of Treated animals. B: Immuno blot and densitometry for PEPCK levels in liver of Control and Treated animals. (n=3), p< 0.05.; C: Immuno blot and densitometry for FOXO1 levels in liver of Control and Treated animals. (n=3), p< 0.05. D-E: Immunoblot and densitometry for P-AKT (Ser-473) (D) and FOXO1 (E) in the liver of Control and Treated animals upon insulin administration, C=Control, C+I= Control animal with Insulin treatment, T=Treated, T+I= Treated animal with Insulin treatment. n=3, p<0.05, *=no significant change as compared to C, †= no significant change as compared to T. (F) FOXO-1 and AR interaction. Liver tissue lysate of insulin administered Control (C+I) and insulin administered Treated (T+I) animals immuno precipitated for FOXO1 and immuno blotted for P-FOXO1 (Ser-256), Androgen receptor (AR) and FOXO1.

insulin resistance, PI3K-AKT independent inhibition of GSK3α has reduced PEPCK level and hence, hepatic gluconeogenesis in liver of Treated animals.

The present study showed that increased hepatic insulin resistance increased FOXO1 level, but this did not lead to increased PEPCK gene expression due to interaction of AR to FOXO1 in the Treated animals. The increased hepatic insulin resistance was counterbalanced by PI3K-AKT independent GSK3 inhibition and AR mediated inhibition of FOXO1, reducing hepatic PEPCK level and hepatic gluconeogenesis. In conclusion, in liver, a dual role of testosterone is observed. On the one hand, testosterone increased insulin resistance in liver and on the other hand interacted with

FOXO1, inhibiting its transcriptional activity and inactivated GSK3α, downregulating PEPCK and reducing gluconeogenesis, which is otherwise upregulated in T2DM. The AR mediated FOXO1 inhibition and PI3K-AKT independent inhibition of GSK3α have down-regulated PEPCK and reduced hepatic glucose output and enhanced glucose homeostasis in liver. Thus, the increased insulin resistance in liver due to testosterone treatment does not worsen T2DM condition, instead improves glucose homeostasis in the Treated animals.

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Molecular mechanism of enzymatic reactions and enzyme-ligand interactions

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Tetramerization and enhanced GMP formation in hGBP1 are allosterically coupled

To examine whether the reduced GMP formation in the chimeras is due to dthe absence of tetramer, we performed analytical gel-filtration assays. Without the analogues, CH1 eluted as a mixture of monomer, dimer and tetramer with tetramer being a considerably smaller amount, whereas CH2 eluted as a mixture of monomer and dimer only. The elution profile for CH1 showed a marginal and higher amount of GppNHp- and GDP. AlF_4-induced dimer, respectively. In contrast, CH2 showed a higher amount of GppNHp-induced dimer, but displayed a relatively lower amount of GDP. AlF_4-induced dimer. More importantly, both chimeras were not capable of forming tetramer with either GppNHp or GDP.AlF_4. These data indicate that exchange of mainly the helical domain of wt-hGBP1 with that of wt-hGBP2 leads to the loss of tetramer formation. Thus, the data imply that the reduced GMP formation in these chimeras is due to their inabilities to form tetramer.

Kinetic studies support tetramerization induced enhanced GMP formation

To further understand this, we performed a detailed steady-state kinetic assay of wt-hGBP1 and chimeras. The k_{cat} for GMP formation in CH1 was decreased by ~ 7.5 fold compared with wt-hGBP1. The $k_{cat}/K_{0.5}$ for GDP formation was also decreased by ~ 47 fold. The decrease in the product formation can be correlated with its inability to form a tetramer. CH2 also showed a similar decrease in the k_{cat} (~83% reduction) for GMP formation, further consistent with the fact that a tetramer is essential for the stimulated GMP formation. Analysis revealed a further interesting difference. Unlike wt-hGBP1, these two chimeras did not show cooperativity (n~1.0-1.1) for GMP formation, indicating

that the reduced GMP formation is associated with lack of cooperativity. Taken together, these results suggest the regulatory role of a tetramer in wt-hGBP1 for enhanced GMP formation through allosteric interactions.

Tetramer is critical for the suppression of Hepatitis C virus proliferation

To check the effect of wt-hGBP1 on HCV (hepatitis C virus) proliferation, wt-hGBP1 expression plasmid was transfected into Rep2a cells harbouring HCV subgenomic replicon. To evaluate the role of its tetramer in antiviral activity, we selected the mutants R48P, T75A and D103L.D108L, where these residues were critical for tetramerization. R48P and D103L.D108L mutants were defective both in the dimerization and tetramerization, whereas T75A was impaired only in the tetramerization. Unlike wt-hGBP1, the expression of either R48P or T75A mutant showed a marginal decrease in the HCV-RNA, whereas the expression of D103L.D108L double mutant almost did not suppress the HCV-RNA. These results indicate that the tetramer of wt-hGBP1 is indeed crucial for the suppression of the virus proliferation.

Aromatic-aromatic contact is essential for retaining the non-conserved motif in a hydrophobic environment

Previous data indicated that Trp159-Asp126 hydrogen bonding interaction is not crucial to retain the motif in a hydrophobic environment and thus possibly not important for its positioning. As observed in MD simulations, Trp159 was in close contact with both His122 and Tyr125. To find out whether these two residues have a role for retention of the motif in a hydrophobic environment, we constructed two double mutants His122Ala/Asp126Ala and Tyr125Ala/Asp126Ala, and performed fluorescence studies as well as dynamic quenching experiments. The results suggest that the interactions of His122 and Tyr125 with Trp159 through their side chains are individually critical for retaining the motif in a hydrophobic environment.

Publications

Original peer-reviewed articles

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Ribonucleases and heat shock proteins: Involvement in host defense

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The work is focused on the following two major themes.

1. Investigation of the role of human ribonucleases, particularly eosinophil ribonucleases, eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) in host defense.
2. Investigation of crucial housekeeping proteins of *M. tuberculosis* for their role in survival and virulence of the pathogen.

Investigation of the involvement of Clp proteases in pathogenic mechanism of *M. tuberculosis*

ClpB is is a heat shock protein that has been known to be involved in thermo-tolerance in various bacteria, however, there is no direct evidence of this in case of *M. tuberculosis*. Homologs of ClpB are known to reactivate aggregated proteins in concert with dnaKJE chaperone machinery. The molecular weight of ClpB monomer is 93 kDa and it has multiple domains. The mycobacterial genome also encodes an N-terminally truncated, 80 kDa isoform of ClpB. In the current study we characterized and compared the full length ClpB protein of *M. tuberculosis* vis-a-vis its N-terminally truncated isoform. Our study shows that ClpB and ClpBΔN exist as hexamers and undergo concentration dependent self-association, in solution. ATP, ADP and AMP-PNP modulate the oligomerization of ClpB and ClpBΔN differently. Both ClpB and ClpBΔN possess nucleotide dependent prevention of protein aggregation activity, nucleotide bound state of the protein affects this activity and is crucial for substrate binding. ClpB-KJE and ClpB(ΔN)-KJE together act as an efficient refoldase. ClpB and ClpBΔN have comparable refolding activity. However, efficiency of refolding depends on the size of aggregated substrate. ClpBΔN is deficient in refolding large aggregates. We have shown that, *in vivo*, ClpB is not essential for survival of *M. tuberculosis* under standard growth

conditions; however, it is essential for *M. tuberculosis* to survive under stress. Loss of clpB results in morphological aberrations in the bacilli during stressed growth conditions.

Investigation of molecular mechanism of biological actions of human ribonucleases and their role in host defense

Alarmins are endogenous mediators of immune system that activate immunity and protect hosts from exogenous danger signals. Eosinophil derived neurotoxin (EDN) has earlier been shown to function as an alarmin. However, its mechanism of action is not clear. In the current study, we carried out a structure-function analysis to investigate the mechanism of EDN's alarmin activity, especially the role of RNase activity and the unique loop L7 in EDN. As reported earlier, we also found EDN to have chemoattractant activity. We observed that ECP also acts as an alarmin, and acts as a chemoattractant for antigen presenting cells. HPR was also found to have chemoattractant activity. RNase activity was found to be crucial for the chemoattractant property of EDN, ECP and HPR. EDN's loop 7 mutants did not show altered chemotactic activity suggesting that this loop is not important for chemotaxis. All the RNases that we have used in this study activate NF-κB via TLR-2 receptors, suggesting that the activation is independent of the level of RNase activity. Interestingly, ECP mutants with reduced basicity showed significantly reduced chemotaxis activity towards DCs, highlighting the importance of basic residues in ECP for chemotaxis.

Publications

Original peer-reviewed articles

1. Singh A, Ubaid-Ullah S, Batra JK (2016) Functional role of putative critical residues in *Mycobacterium tuberculosis* RNase P protein. *Int J Biochem Cell Biol.* **78**:141-148.
2. Zaidi S, Haque MA, Ubaid-Ullah S, Prakash A, Hassan MI, Islam A, Batra JK, Ahmad F (2017) Denatured states of yeast cytochrome *c* induced by heat and guanidinium chloride are structurally and thermodynamically different. *J Biomol Struct Dyn.* **35**:1420-1435.
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Structural and functional studies of *Mycobacterium tuberculosis* proteins

Bichitra K. Biswal

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We have been working on two projects involving structural and functional aspects of proteins from *Mycobacterium tuberculosis (M.tb)*, the organism that causes tuberculosis (TB) in humans. The first one aims at understanding the 3D structures and 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 *M.tb* membrane associated proteases modulate host factors.

Mtb biosynthesizes 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,* mainly in the context of deciphering the molecular mechanisms underlying their functions 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 triazole scaffold compounds are competitive inhibitors of HisB.

Hitherto, the enzyme that catalyses dephosphorylation of L-histidinol phosphate to L-histidinol, the eighth step of this pathway was not known. Through bioinformatics approach combined with biochemical study we have identified the enzyme, referred here as HisN and have elucidated its 3D structure. The biological functional unit of HisN is a dimer. Analysis of its 3D structure showed that the overall structure of a mononer (260 amino acids) folds into two

distinct structural domains, N and C-terminal domains (Figure 1). Notably, these domains are connected by a 20-residue long loop. The N-terminal domain consists of residues 2-130. Its tertiary structure is comprised of two long alpha helices followed by a six-stranded anti-parallel β-sheet. The C-terminal domain comprised of residues 150-260 and folds into a globular structure consisting a five-stranded anti-parallel β-sheet sandwiched between six α-helices. Structure of HisN-substrate complex was determined. The underlying mechanism of its action is being delineated. With regard the work on membrane proteins, structure of a truncated version of a probable serine protease (Rv1223) was determined and its mechanism of action is being elucidated.

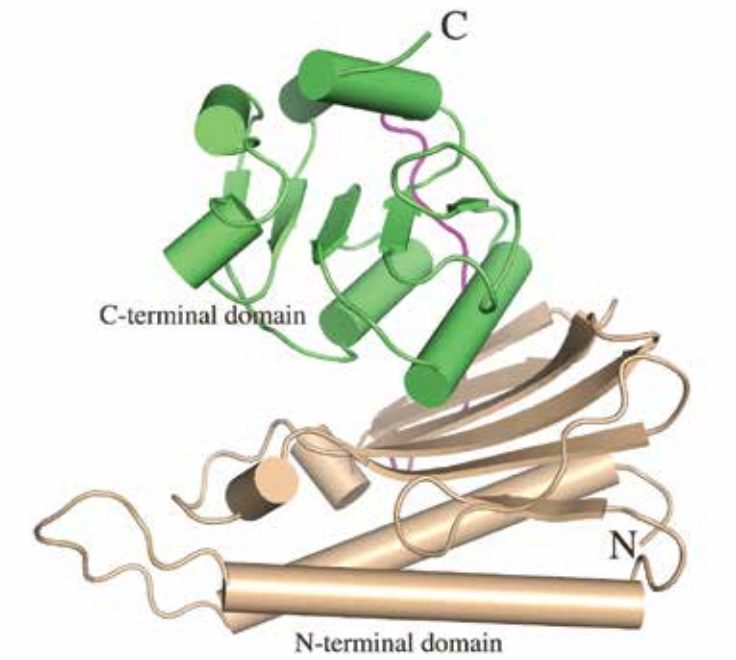

Figure 1. Cartoon representation of the 3D structure of HisN.

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

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The objectives of the various projects are to investigate, whether the combination of knowledge-based and *ab initio* computational approaches can be used for (1) *in silico* identification of secondary metabolites by genome mining (2) deciphering substrate specificity of various peptide recognition modules (PRMs and genome wide prediction of their interaction networks (3) structure based analysis of RNA-protein and DNA-protein interactions associated with gene regulation.

A. Computational methods for prediction of chemical structures of RiPPs

Ribosomally synthesized and post-translationally modified peptides (RiPPs) constitute a rapidly growing class of natural products with diverse structures and bioactivities. We have developed a novel computational method for deciphering chemical structures of RiPPs by genome mining. Our method derives its predictive power from machine learning based classifiers, trained using a well curated database of more than 500 experimentally characterized RiPPs. It uses SVM to distinguish RiPP precursors from other small proteins and classify the precursors into twelve sub-classes of RiPPs. For classes like lanthipeptide, cyanobactin, lasso peptide and thiopeptide, our method can predict leader cleavage site and complex cross-links between post-translationally modified residues starting from genome sequences. Our method can identify correct PTM and cross-link pattern in a query RiPP core peptide from among a very large number of combinatorial possibilities with high sensitivity, specificity, accuracy and precision. This computational approach has been implemented in a web based software RiPPMiner, which is available at http://www.nii.ac.in/rippminer.html.

B. Genome wide prediction of interaction networks of PDZ domains and kinases

Straight forward extension of such sequence or structure based methods for genome wide prediction of interaction networks results in huge number of false positives, because various cellular contexts of interaction (e.g. coregulation, coexpression, colocalization etc) are not taken into considerations. Our earlier analysis of domain specific kinase-substrate relationships had revealed that calculation of interaction propensities based on co-occurrence of other PFAM domains in interacting proteins helps in implicitly incorporating context information and significantly reduces false positives. Now

we have developed a computational method for genome scale prediction of site specific kinase-substrate relationships and interaction partners of PDZ domains by combining information about domain composition of the interacting proteins with sequence information of the PRM (i.e. kinase or PDZ) and its recognition site. Benchmarking studies indicate superior performance of our approach compared to sequence/domain based methods like PDZPepInt and phosD with ROC-AUC value of ~0.9 (PDZ) and 0.82 (Kinase).

C. MD simulations on CTCF in complex with DNA motifs

CTCF is the key architectural protein which aids in long range interactions in genomic DNA via chromosomal looping and plays a crucial role in promoter-enhancer interaction and insulator function. Different functional roles have been implicated for binding of CTCF to low or high occupancy sites. In order to decipher the structural basis of CTCF code, we have used double helical DNA (23 base pairs) bound crystal structure of a protein containing artificial six zinc finger domains as structural template. Explicit solvent MD simulations have been carried out for 1 μs using ff14SB and parmbsc0 force fields for protein and DNA respectively. The simulation trajectory has been analyzed for changes in intermolecular hydrogen bonding, backbone dihedrals of DNA, DNA bending and curvature. After characterizing the conformational flexibilities of the structural template, CTCF-DNA complexes corresponding to high occupancy and low occupancy CTCF binding sites will be modelled and analyzed to identify specificity determining residues (SDR) responsible for CTCF binding to DNA.

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Original peer-reviewed articles

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

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Our laboratory strives to explore and unravel structural and functional roles of glycoconjugates in biological systems. Towards this end, we design, synthesize, and develop carbohydrate-based small molecules as probes and inhibitors of glycosylation pathways. We investigate (A) inhibition of mucin-type *O*-glycosylation (MTOG) *in vitro* in mammalian cells, *ex vivo*, and in mice towards potential modulation of autoimmune diseases and (B) development of non-invasive tools for modulation of glycans of the brain in mice for both imaging and inhibition.

A. Modulation of glycoforms of cluster of differentiation (CD) antigens.

We have investigated inhibition of MTOG induced by Ac_5GalNTGc, in a thiol-dependent manner, along with a panel of GalNAc analogues. Upon treatment with Ac_5GalNTGc, but not corresponding *N*-acetyl and *N*-glycolyl analogues, EL4 (mouse thymoma) cells showed drastic reduction in *Maackia amurensis* lectin – II (MAL-11), increase in Vicia villosa agglutinin (VVA), and no change in Sambucus nigra agglutinin (SNA) and peanut agglutinin epitopes (PNA). RAW264.7 (mouse macrophage) cells showed reduction in MAL-II and PNA epitopes, moderate increase in VVA, and no change in SNA epitopes. This suggested thiol-dependent hyposialylation and exposure of Tn-antigen on cell surface glycoproteins in EL4 and RAW 264.7 cells.

***Glycoform modulation of CD43*:**

Similar to human CD43, mouse CD43 is also known to carry 80-90 *O*-glycans (**Figure 1A**). EL4 and RAW264.7 cells express 115 (anti-CD43-S7 epitope, neuraminidase sensitive) and 130 kDa (anti-CD43-1B11 epitope) glycoforms of CD43, respectively. A drastic reduction in CD43-S7 epitopes was observed in EL4 cells treated with Ac_5GalNTGc, but not in controls; while CD43-M19 (glycan independent, binds to C-terminal) blots showed faster moving bands at lower molecular weight indicating inhibition of MTOG. On the other hand only a moderate reduction in CD43-1B11 epitopes was observed in RAW264.7 cells, which is attributable to the presence of higher oligosaccharides on CD43 in macrophages. Extracellular domain of CD43 contains a total of 93 potential MTOG sites (46 Ser and 47 Thr). Soluble CD43-Fc-His was purified from Jurkat cells and digested with both trypsin and Glu-C. Glycopeptides were analyzed on a high-resolution nano-LC-ESI-MS/MS system using high energy collision dissociation product-dependent electron transfer dissociation. Results revealed that on an average 71 sites were occupied in CD43-Fc-His.

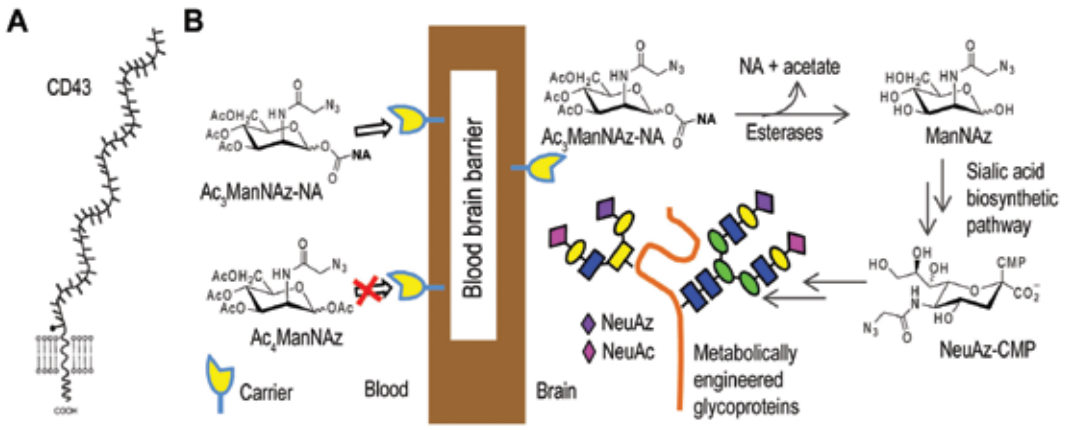

Figure 1. Applications of metabolic glycan engineering. A) Schematic representation of CD43 (leukosialin / sialophorin) carrying 80-90 mucin type *O*-glycans and one membrane proximal *N*-glycan. Treatment with Ac_5GalNTGc induces drastic hyposialylation and hypo-glycosylation of CD43. **B**) Carbohydrate-neuroactive hybrid (CNH) strategy for piggy backing of non-natural hexosamine analogues to achieve modulation of glycoconjugates of the brain. NA, neuroactive moiety.

B. Modulation of glycans of the central nervous system (CNS) across blood-brain barrier (BBB):

Pioneering studies by Reutter, Bertozzi, and others have shown expression of non-natural NeuAc expression in glycoproteins of heart, kidney, and liver, but not in brain. To overcome the inability of hexosamine analogues to reach CNS, we envisaged the carbohydrate-neuroactive hybrid (CNH) strategy for delivery to brain across BBB (**Fig. 1B**). Hybrids of ManNAc analogues with nicotinate and valproate were found to induce expression of NeuAz carrying sialoglycoproteins in brain while non-hybrid molecules did not. Using *N*-butanoyl derivatives and CNH strategy, modulation of polysialic acid (polySia) on neural cell adhesion molecule (NCAM) *in vivo* was accomplished. Western blots of brain lysates using anti-polySia (12F8) showed significant decrease while the anti-NCAM (OB11) blots showed the polypeptide levels to be unaffected. Such modulation of polySia could have varied applications in the study of learning, memory, and brain tumors.

Publication

Original peer-reviewed article

1. Shajahan A, Parashar S, Goswami S, Ahmed SM, Nagarajan P, Sampathkumar SG* (2017) Carbohydrate-Neuroactive Hybrid Strategy for Metabolic Glycan Engineering of the Central Nervous System *in Vivo*. *J Am Chem Soc* **139**: 693-700.

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Role of carbohydrates in modulating the structure and function of glycopeptides

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The project is aimed for 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.

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

Antimicrobial peptides (AMPs) are key players of innate immunity. Amongst various classes of AMPs, proline rich AMPs from insects enjoy special attention with few members of this class bearing *O*-glycosylation as post-translational modification. Drosocin, a 19 amino acid glycosylated AMP is a member of proline rich class, synthesized in the haemolymph of *Drosophila melanogaster* upon bacterial challenge. We chemically synthesized drosocin carrying disaccharide (β-Gal(1→3) α-GalNAc) and compared its structural and functional properties with another naturally occurring monoglycosylated form of drosocin i.e. α-GalNAc-drosocin as well as with non-glycosylated drosocin. The disaccharide containing drosocin exhibited lower potency compared to mono glycosylated drosocin against all the tested Gram negative bacteria, suggesting role of distal sugar or increase in sugar chain length on the activity. Cytotoxic properties of drosocin were not altered due to increase in sugar chain length. Circular Dichroism studies failed to demonstrate the differential effect of sugars on the overall peptide conformation. In addition, we have also studied the effect of differentially glycosylated drosocins on two cytokines secreted by murine macrophages or LPS stimulated macrophages. Our results suggest that all the drosocin forms could not stimulate the secretion of TNF-α and IL-6 and even did not modulate LPS-induced levels of TNF-α and IL-6 in macrophages.

Indolicidin which belongs to the cathelicidin family, has been shown to have broad spectrum of antimicrobial activity. However, at the bactericidal concentrations, it exhibits substantial toxicity towards mammalian

erythrocytes and other cells. It is therefore of interest to design the glycosylated analogs of indolicidin for investigating the sugar potential in reducing its cytotoxic property and structure-activity relationship of designed analogs of indolicidin. We have shown that the designed glycosylated analog exhibited the antibacterial and immunomodulatory activities similar to indolicidin, however it was non-toxic towards erythrocytes and murine macrophages. To analyze the structural effects of glycosylation on peptide backbone, the comparative conformational properties of indolicidin and its glycosylated analog were studied by CD spectroscopy in PB, 50 %TFE and 10mM SDS. It was observed that the gross conformational characteristics of glycosylated analog resemble the native indolicidin in different environment. To examine the precise effect of glycosylation on the structure of indolicidin analog, comparative NMR studies of indolicidin and its glycosylated analog were carried out. The 2D homonuclear spectra, TOCSY and NOESY, in DPC were acquired at 37^{o}C. The reasonable differences in chemical shifts (>1ppm) were observed for H^{N} values of W^{4},W^{6},W^{8}, W^{11} and R^{12} among indolicidin and its glycosylated analog. The observed differences between the chemical shift values of amide protons of indolicidin and glycosylated peptide suggest an effect of sugar on the backbone conformation of the glycosylated analog. Detailed structure characterization of glycosylated peptide is in progress.

Publication

Original peer-reviewed article

1. Vashisth S, Kumar A, Kaur KJ, Salunke DM (2016) Antibodies can exploit molecular crowding to bind new antigens at noncanonicalparatope positions. *Chemistry Select* **1**: 6287-6292.

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Elucidate chemico-cellular processes underlying pathological outcomes in tuberculosis and vitiligo

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My laboratory is interested to elucidate mechanistic and spatiotemporal coherence of cellular processes involved in two distinct pathological diseases – Tuberculosis (TB) and Vitiligo.

The objectives of the studies proposed are:

i. Delineating networks and pathways underlying biosynthesis or degradation/recycling of lipidic metaboilites in mycobacteria

ii. Identify factors regulating melanogenesis and decoding the spatiotemporal coherence associated with melanocyte-keratinocyte biology in vitiligo subjects

In the previous year, we reported skin microbiome analysis from lesional and non-lesional skin from vitiligo patients. Our study revealed dysbiosis in the diversity of microbial community structure in lesional skin of vitiligo subjects. Although individual specific signature was dominant over the vitiligo-specific microbiota, a clear decrease in taxonomic richness and evenness was noted in lesional patches.

Classical autophagy protein LC3B facilitate melanosome movement on cytoskeletal tracks

Macroautophagy is a dynamic and inducible catabolic process that responds to a variety of hormonal and environmental cues. Previous studies showed presence of the autophagic marker LC3B on melanosomes, however, the biological significance could not be established and it was not clear whether melanosomes are cleared through the process of selective autophagy. Our immunofluorescence analysis showed a punctate colocalization for LC3B on melanosomes. After confirming this colocalization with several other markers, quantitative analysis of many cells revealed that about two-thirds of the melanosomal population in each cell was labelled with LC3B. The remaining 30% of melanosomes in melanocytes did not colocalize with LC3B. By performing serial z-stack optical sections of ~500 nm, we observed an interesting trend of enrichment of LC3B-labelled melanosomes in the center of the cell and absence at the periphery. Electron microscopy studies established that increase in LC3B flux is not leading to autophagic vesicles of melanosomes and that LC3 had some non-canonical role in melanosome biology.

Melanosome biogenesis and maturation requires sequential delivery of proteins through vesicular trafficking. We therefore examined and ruled out the role of increased LC3B flux in melanosome formation. Interestingly, the LC3B knockdown

(~65%) in melanocytes revealed a perinuclear clustering of melanosomes and there was a significant absence of melanosomes from the dendritic tips. Since LC3 was first identified as a light chain (LC-3) of microtubule-associated protein 1 (MAP1) and 2 (MAP2), we probed whether this melanosome movement defect is not a consequence of microtubule (MT) instability. By live imaging studies, we showed that there was no effect of LC3 knocks down on mitochondrial movement. This indicated that the trafficking defect is specific to melanosomes with LC3B knockdown. Melanosomes are understood to primarily originate from the center of the cell and traverse on microtubule (MT) tracks to reach the distal ends. At the dendritic ends melanosomes crossover to actin filaments that are spatially enriched at the cellular periphery. Immunostaining of melanosomes with tubulin and actin showed that melanosomes at the cell center localized on MT whereas at the dendritic tips melanosomes were preferentially present on actin. Inhibitor analyses with microtubule- and actin-disrupting agents on melanosome distribution and pull-down studies confirmed the role of LC3B in melanosome mobilization on microtubules.

As we continue to enrich our knowledge, we would like to define trajectory of vitiligo disease progression that will eventually make it possible to find cure for the debilitating chronic complex disease vitiligo.

Publications

Original peer-reviewed articles

1. Dani P, Patnaik N, Singh A, Jaiswal A, Agrawal B, Kumar AA, Varkhande SR, Sharma A, Vaish U, Ghosh P, Sharma VK, Sharma P, Verma G, Kar HK, Gupta S, Natarajan VT, Gokhale RS, Rani R (2017) Association and expression of antigen processing gene PSMB8 coding for Low Molecular Mass Protease 7 (LMP7) with Vitiligo in North India: case-control study. *Br J Dermatol.* DOI: 10.1111/bjd.15391.
2. Vij M, Grover R, Gotherwal V, Wani NA, Joshi P, Gautam H, Sharma K, Chandna S, Gokhale RS, Rai R, Ganguli M, Natarajan VT (2016) Bio-inspired functionalized melanin nano-variants with a range of properties provide effective color matched photo-protection in skin. *Biomacromolecules* **17**: 2912-2919.
3. Singh VP, Motiani RK, Singh A, Malik G, Aggarwal R, Pratap K, Wani MR, Gokhale SB, Natarajan VT, Gokhale RS (2016). Water Buffalo (Bubalus bubalis) as a spontaneous animal model of Vitiligo. *Pigment Cell Melanoma Res* **29**:465-469.
4. Kinatukara P, Patel KD, Haque AS, Singh R, Gokhale RS, Sankaranarayananan R (2016). Structural insights into the regulation of NADPH binding to reductase domains of nonribosomal peptide synthetases: A concerted loop movement model. *J Struct Biol.* **194**:368-374.

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Kritee Mehdiratta
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Amarnath Prasad
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Jyotsna

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

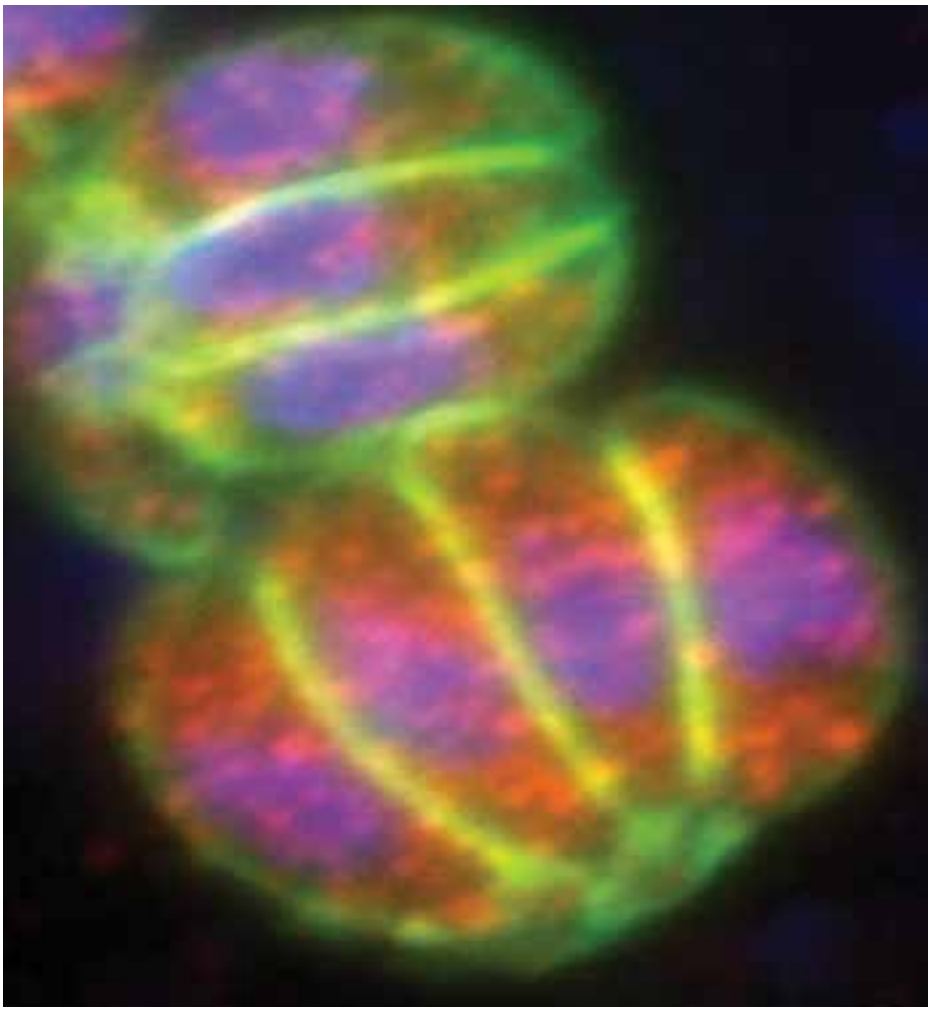
Vidya Raghunathan

Leishmania species uses multiple isoforms for enzymes of the energy pathway, one of which is phosphoglycerate kinase or PGK. Using NMR, we looked at the structure of the 62-residue extension of the entire C-terminal domain of PGKC-Lmex by cloning in *E. coli* BL21 (RP) strain. The protein was expressed and western blotting shows the presence of a band after purification by metal affinity chromatography at the molecular weight expected for the peptide of about 7 kDa. We cloned PGKC-Lmex in *E.coli*. These efforts target towards drug developments at the glycosome level.

We used dynamic methods to determine the structure and conformational studies of PGKC-*Lmex* and PGKB-*Lmex* and reported the higher ATP affinity with the *Leishmania* enzymes and higher ADP affinity for the 3-PG ligands. Furthermore, the homology based modeling showed a base structure for PGKC-*Lmex* and PGKB-*Lmex* by using the softwares namely, SWISS-MODEL, ClusPro 2.0, Protein-Protein Docking, Jpred 4 and Chimera 1.10.1. The template used was of *T. bruceiphosphoglycerate kinase* whose crystal structure is available in PDB. Docking software was used to deduce the structural relationship between the core protein and the 63-mer extension (excluded in the template-based homology modeling). Biochemical data on peptide-protein interaction in which, we have used synthetic peptide mixtures (same as that used in the work published in MBP, 2012) with wild-type recombinant PGKB-*Lmex,* narrowed down the conformational scape and give a model for whole PGKC-*Lmex*.

The main objectives are as follows:

1. Expression, purification and determination of specific activities of PGKB-*Lmex* and PGKC-*Lmex.*
2. The steady state kinetics studies of PGKB-*Lmex* and PGKC-*Lmex.*
3. Comparison between pH optimum activity of PGKB-*Lmex* and PGKC-*Lmex* and enzyme inhibition by salt and suramin.
4. ^{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.
5. Peptide based studies of glycosomal membrane association of PGKC-*Lmex*. The peptides used in these studies were evaluated as useful models to understand the structural basis of the biochemical differences between PGKC-*Lmex* and PGKB-*Lmex*.
6. Using promastigote and amasitoge cultures of *Leishmania spp* for metabolomemapping.Theconcentration of specific metabolites in the cell at a particular time was monitored at the micromillimolar level. The metabolites detected were alanine, lactate, acetate, pyruvate, succinate, glycerol, urea, CO_2, oxalate, valine, glutamine and arginine.



Toxoplasma parasite. Autophagy-related protein TgATG18 (red), SAGI (green)

GENE REGULATION

Cellular and molecular biology of human cancer

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Triple negative breast cancer (TNBC) is most difficult to treat and the prognosis is very poor. Recently, we demonstrated the association of sperm associated antigen 9 (SPAG9) expression in breast cancer tissue samples. Present investigation was carried out to understand role of SPAG9 in breast cancer tumorigenesis in TNBC cell line MDA-MB-231.

SPAG9 expression and cellular proliferation in breast cancer cell lines

SPAG9 protein localization analysis in BT-474, MCF7, MDA-MB-231 and SK-BR-3 breast cancer cells showed predominant cytoplasmic expression with localization in endoplasmic reticulum, Golgi bodies and mitochondria. However, SPAG9 did not localize with nuclear envelope (Fig. 1A). Further, western blot analysis revealed significant reduction in SPAG9 expression employing SPAG9 shRNA1 and SPAG9 shRNA2 (Fig. 1B). Knockdown of SPAG9 also resulted in cell cycle arrest in MDA-MB-231 cell lines. Western blotting analysis revealed significant decrease in cell cycle molecules and upregulation of tumor suppressor protein, p21. These results suggest that SPAG9 ablation results in cell cycle arrest of breast cancer cells. Cellular senescence assay revealed that the percentage of senescent cells was significantly higher with SPAG9 shRNA as compared to controls. Enhanced expression of the level of DcR2 was observed after knockdown of SPAG9 as compared to NC shRNA transfected cells. Further our *in-vivo* studies demonstrated significant reduction in tumor size in shRNA treated tumor as compared to controls. IHC analysis of tumor serial sections revealed significantly enhanced immuno-reactivity of p21 and decreased immuno-reactivity of cell cycle molecules in SPAG9 shRNA treated mice as compared controls. Specifically, cyclinB1,

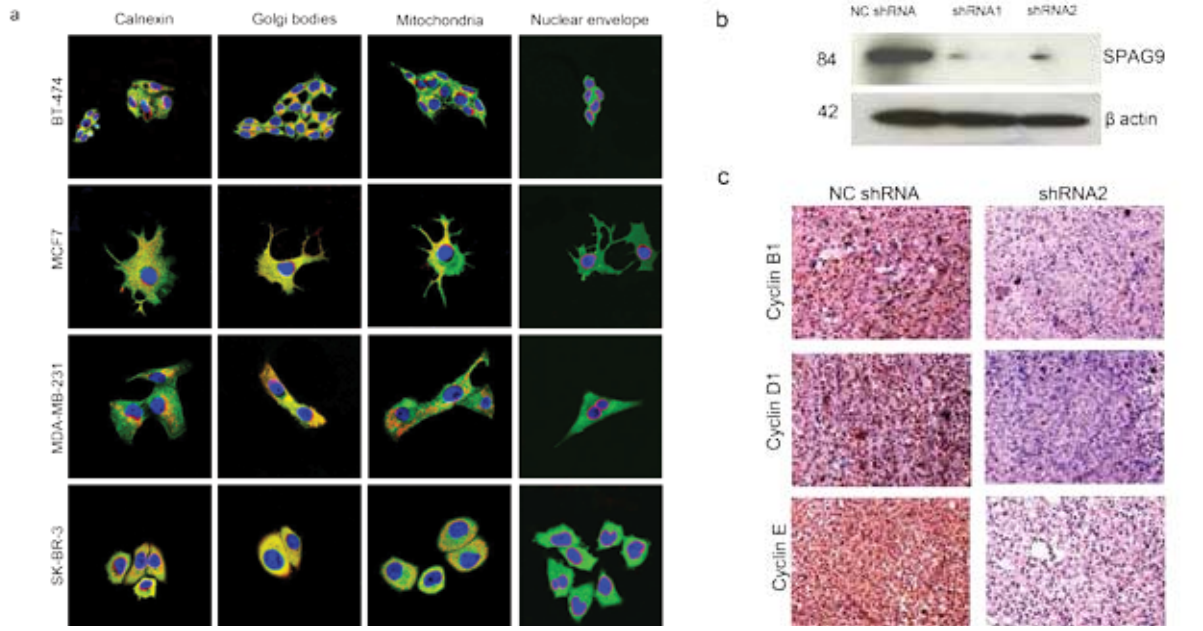

Figure 1. SPAG9 expression in different breast cancer cells. a) The confocal images exhibit the co-localization of SPAG9 in endoplasmic reticulum, golgi bodies and mitochondria (orange-yellow staining), whereas, no co-localization was observed in nuclear envelope. b) Western blot analysis show significant SPAG9 protein ablation in shRNA1 and shRNA2 transfected cells as compared to NC shRNA transfected cells. c) Representative images of IHC analysis of tumor serial sections of animals injected with SPAG9 shRNA2 show reduced immuno-reactivty of cell cycle molecules.

cyclinD1 and cyclin E showed decreased reactivity in SPAG9 ablated tissue sections (Fig. 1C).

Effect of SPAG9 shRNA on apoptosis

Various biochemical assays JC1 assay, TUNEL assay, annexin V-FITC staining and M30 assay showed significant increase in apoptosis in SPAG9 shRNA transfected cells as compare to NC shRNA. Scanning electron microscopy (SEM) validated increase in apoptosis in SPAG9 shRNA transfected MDA-MB-231 cells (Fig. 2C). Blebbing, holes and apoptotic bodies were seen in cells transfection with SPAG9 shRNA but not in controls (Fig. 2C). Ablation of SPAG9 altered molecular pathways involved in cellular proliferation, senescence, apoptosis and EMT. Furthermore, IHC analysis of TNBC human xenograft validated our *in-vitro* findings. Collectively, we demonstrated that SPAG9 protein is a key molecule which plays important role at molecular level in tumor growth by regulating apoptosis, cell cycle and EMT pathways. Our study has laid the foundation wherein SPAG9 could be a potential molecule for developing as therapeutic target for TNBC treatment.

Human clinical trials in cervical cancer patients

Dendritic cell based vaccine- Phase II Human clinical trials in cervical cancer patients stage IIIb employing therapeutic grade Recombinant SPAG9 is underway in joint collaboration with Cancer Institute, Adyar, Chennai and National Institute of Immunology, New Delhi.

1. **Early Detection and Diagnosis: [NCI-AIIMS, NII, Cancer Institute Chennai]**

   The goal is to advance medical research and improve patient outcomes by discovering biomarkers (indicators) for multiple types of cancer. The early detection of cancer is crucial for its ultimate control and prevention. Large scale validation of SPAG9 will be carried out in NCI-AIIMS and Cancer Institute, Chennai for ovarian, breast and prostate

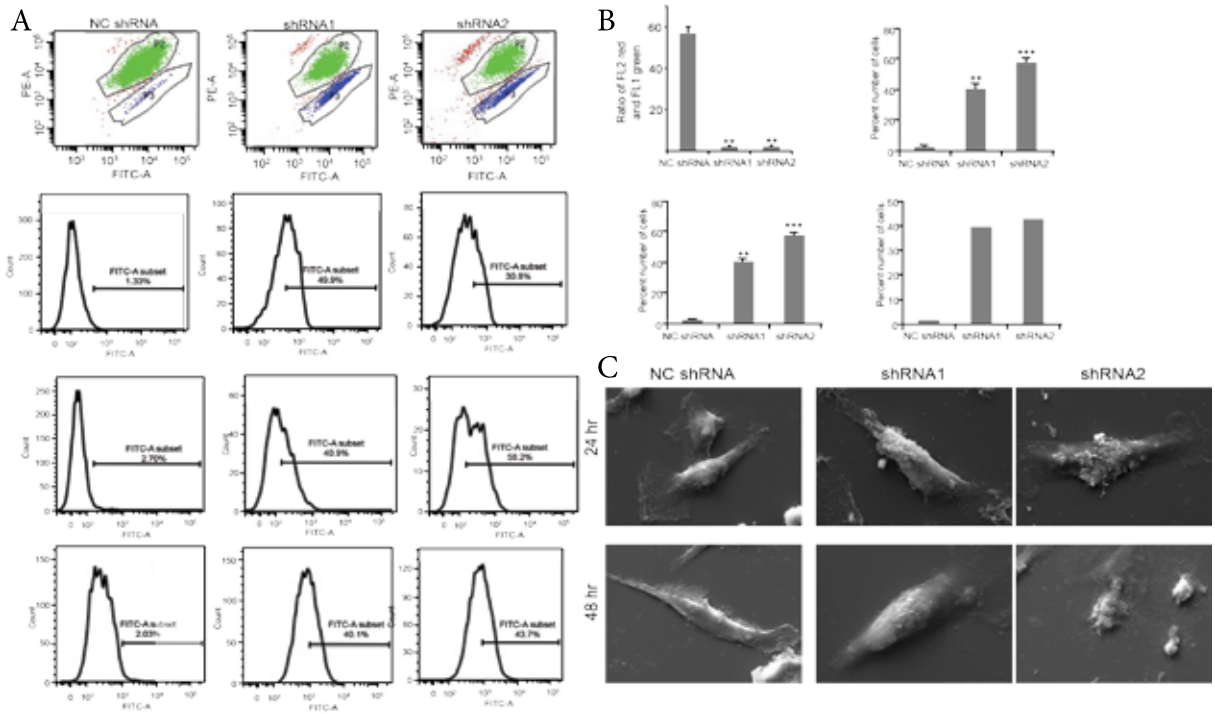

Figure 2: FACS analysis shows SPAG9 ablation upregulated apoptosis in MDA-MB-231 cells by JC1 staining, TUNEL assay, annexin V staining and M30 assay (Fig. 2A). Histogram depicts percentage of cells with decreased mitochondrial membrane potential, increased DNA damage, enhanced phosphatidyl serine expression and increased caspase activation in SPAG9 shRNA1 and shRNA2 cells transfected and control (Fig. 2B). Photomicrographs of SEM show MDA-MB-231 cells undergoing apoptosis after SPAG9 ablation (Blebbing and disordered structure of cells (Fig. 2C).

cancer. Our aim is to use SPAG9 molecules for detecting cancers at their earliest stages. This will mean that current or future treatment strategies will have a higher probability of truly curing the disease.

2. **Dendritic cell based vaccines: NCI-AIIMS, Cancer Institute, Chennai**

Human cervical cancer trials are under way using therapeutic grade recombinant SPAG9 for DC based vaccine. The trials SOP have been generated. The patient's recruitment has been initiated in January 2017. Dendritic cells (DCs) are potent antigen-presenting cells that control primary and secondary immune responses to various exogenous antigens. Tumor associated antigens discovered in our studies will be primed with DCs and explored for their immunotherapeutic potential.

3. **Therapeutic and Prophylactic Immunization [NII] Tumor Vaccines:**

Active immunotherapy with tumor antigens is an attractive option for cancer therapy. However, this approach is hampered by the identification of novel tumor-associated antigens which are associated with early tumor progression, and by the immunosuppressive milieu associated with cancer growth. SPAG9 has emerged as a promising candidate for Prophylactic and therapeutic vaccination against cancer because of its peculiar expression profile in various malignancies and humoral and cellular immune responses. The implementation of these insights in the clinical setting and the completion/conduction of human randomized trials will ultimately help to define the translational aspects of these cancer vaccines against malignancy.

Publications

Original peer-reviewed articles

1. Jagadish N, Parashar D, Gupta N, Agarwal S, Suri V, Kumar R, Suri V, Sadasukhi TC, Gupta A, Ansari AS, Lohiya NK, Suri A (2016) Heat shock protein 70-2 (HSP70-2) is a novel therapeutic target for colorectal cancer and is associated with tumor growth. *BMC Cancer* **16**: 561-574.
2. Jagadish N, Gupta N, Agarwal S, Parashar D, Sharma A, Fatima R, Topno AP, Kumar V, Suri A (2016) Sperm associated antigen 9 (SPAG9) promotes the survival and tumor growth of triple-negative breast cancer cells. *Tumor Biol* **37**:13101 – 13110.
3. Jagadish N, Agarwal S, Gupta N, Fatima R, Devi S, Kumar V, Suri V, Kumar R, Suri V, Sadasukhi TC, Gupta A, Ansari AS, Lohiya NK, Suri A (2016) Heat shock protein 70-2 (HSP70-2) overexpression in breast cancer. *J Exp Clin Cancer Res.* **35**:150-164.

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

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Tuberculosis has been a long-standing problem in our country and due to the emergence of drug-resistant strains, the search for new drug targets continues. Understanding the cellular processes of the pathogen is central to finding effective means of therapeutic intervention. Eukaryotic-like serine/threonine protein kinases (STPKs) in *M. tuberculosis* regulate a number of cellular processes including cell division. There are 11 eukaryotic-like STPKs in *M. tuberculosis*, and we have worked towards analyzing the functional roles of the phosphorylation events mediated by these kinases. Investigating the signaling events in the pathogen contributes significantly towards understanding the biological events that are coupled to the manifestation of the disease.

Two out of the eleven STPKs found in *M. tuberculosis* namely Protein kinase A (PknA) and Protein kinase B (PknB) are essential for the bacterial survival *in vitro* and *in vivo*. The essential kinases PknA and PknB regulate cell growth/division and cell wall synthesis through the phosphorylation of a myriad of substrates involved in mycolic acid synthesis, peptidoglycan synthesis, cell metabolism, transcription and translation. Over expression of PknA or PknB in mycobacteria results in elongated and bulged cells respectively, with excessive overexpression of PknB resulting in cell death. PknA and PknB have four distinct parts: an N- terminal kinase domain, followed by a juxtamembrane domain that is in turn connected to the extracytoplasmic region through a short transmembrane domain. The intracellular kinase domain of PknB is by itself catalytically active, and kinase activity is regulated through auto-phosphorylation of specific residues in the activation loop. While PknA has a short extracellular region without any defined domain structure, the extracytoplasmic region of PknB comprises of four tandem penicillin-binding protein and serine/threonine kinase-associated (PASTA) domains.

Though the kinase domain of PknB is active *in vitro*, it fails to rescue defects due to PknB depletion in the *M. tuberculosis* conditional Rv-cd-B mutant. The extracytoplasmic PASTA domains of PknB have been found to be necessary for the growth of *M. tuberculosis in vitro,* and deletion of the fourth PASTA domain is sufficient to compromise the bacterial growth. The PASTA domains of PknB are necessary and sufficient for appropriate localization of PknB in *M. smegmatis*.

These domains are thought to play a role in the recognition of muropeptides, which are building blocks of peptidoglycan. Turapov et al have shown that over-expression of PknB extracellular region in mycobacteria leads to delayed re-growth phenotype. The PASTA domains over-expressing mycobacterial cells are more resistant to β-lactam antibiotics and show aberrant expression of many cell wall synthesis associated genes.

Previously we have reported that the fourth PASTA domain (PASTA4) is absolutely essential for the function of PknB in *M. tuberculosis*. It has been speculated that the interaction of PASTA domains with the muropeptide ligands results in dimerization, which in turn results in activation of PknB. However, till date the hypothesis has not been experimentally tested *in vivo*. Further the importance of PASTA domain specificity, criticality of domain length, and the specific residues in the PASTA domain that play a role in PknB mediated signaling have not been investigated. We aimed to identify the specific PASTA domain residues that are necessary for interaction and PknB signaling. The data obtained suggests that both, the total length of the four domains, as well as the particular presence of the PASTA4 domain, are vital for PknB signaling. Using an *in silico* approach we identified four putative residues in the linker region between PASTA3 and PASTA4: $Ser_{556,}$ $Lys_{557,}$ Asn_{559} and $Gln_{560,}$ which could be involved in muropeptide binding. Mutational and complementation analyses suggested that PASTA3-4 linker mutants were significantly compromised in their ability to support mycobacterial growth. Using phospho-specific antibodies to the activation loop as well as mass spectrometry analysis, we found that activation loop phosphorylation is refractory to PASTA3-4 linker mutations. Taken together, these results suggest that the extracellular domain-mediated regulation of PknB signaling is not through the modulation of activation loop phosphorylation events.

Publications

Original peer-reviewed articles

1. Sengupta S, Naz S, Das I, Ahad A, Padhi A, Naik S, Ganguli G, Patnaik K, Raghav SK, Nandicoori V, Sonawane A (2017) Mycobacterium tuberculosis esxL inhibit MHC-II expression by promoting hypermethylation in class-II transactivator loci in macrophages. *J Biol Chem.* **292**:6855-6868.
2. Sharma AK, Arora D, Singh LK, Gangwal A, Sajid A, Molle V, Singh Y, Nandicoori VK (2016) Serine/threonine protein phosphatase PstP of *Mycobacterium tuberculosis* is necessary for accurate cell division and survival of pathogen. *J Biol Chem.* **291**:24215-24230.
3. Jhingan GD, Kumari S, Jamwal SV, Kalam H, Arora D, Jain N, Kumaar LK, Samal A, Rao KV, Kumar D, Nandicoori VK (2016) Comparative proteomic analyses of avirulent, virulent and clinical strains of *M. tuberculosis* identifies strain-specific patterns. *J Biol Chem.* **291**:14257-14273.
4. Sharma G, Sowpati DT, Singh P, Khan MZ, Ganji R, Upadhyay S, Banerjee S, Nandicoori VK, Khosla S (2016) Genome-wide non-CpG methylation of the host genome during *M. tuberculosis* infection. *Sci Rep.* **6**: 25006.

Patent

1. 3167/DEU2015 Applied on 1st October 2016. Depletion of *M. tuberculosis* GlmU from infected murine lungs effects the clearance of the pathogen.

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

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Using a combination of genetics, molecular biology and genomics in the model system *Caenorhabditis elegans,* we aretrying to decipher signalling events that culminate in alterations in gene expression during aging.

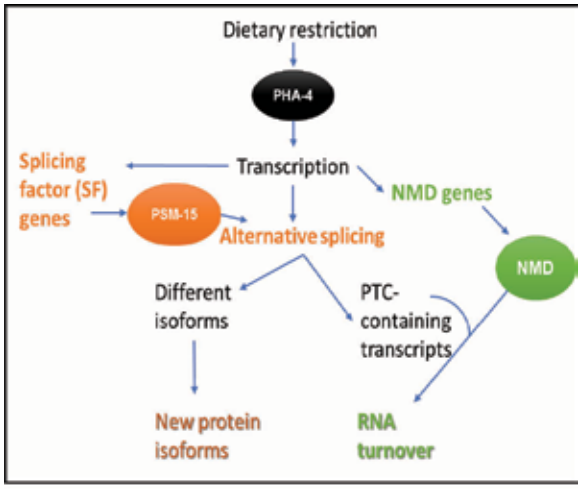
A. Deciphering the coordinate regulation of genes downstream of the IIS pathway

In *C. elegans*, the IIS and DR pathways act on FOXO/DAF-16 and FOXA/PHA-4, respectively. However, these pathways often synergize, suggesting a common downstream player. Using ChIP-seq and quantitative expression analysis, we had earlier shown that chromatin modifier ZFP-1 as well as its interactor GFL-1 works downstream of the Insulin-IGF-1-like (IIS) pathway under direct transcriptional control of FOXO/DAF-16. Now, we found that ZFP-1/GFL-1 are also direct targets of FOXA/PHA-4. On knocking down ZFP-1 or GFL-1 in *eat-2(ad1116)*, the expression of PHA-4 target genes increase. Thus, similar to IIS pathway, PHA-4 regulates ZFP-1/GFL-1 that help in fine-tuning the amplitude and duration of gene expression during DR. Finally, we show that *zfp-1* and *gfl-1* are required for DR-mediated longevity. Together, our study established ZFP-1 as a novel post-transcriptional integrator of IIS and DR pathways.

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

In DR worms, the expression of Non-sense-mediated decay (NMD) pathway genes are upregulated. We knocked down a key component of NMD, SMG-2/UPF1 and found that DR failed to increase life span. Using transcriptomics, we identified transcripts that are targeted by NMD during DR; these are involved in splicing, innate

immunity and metabolism. Finally, we showed that the DR-specific transcription factor PHA-4/FOXA transcriptionally regulates the AS-NMD genes. Together, this study shows that during DR, AS-NMD may thus provide an energetically favourable level of dynamic gene expression control.



A model showing regulation of AS-NMD during DR.

C. Involvement of novel kinases in DR

Since p38 MAPK pathway was found to upregulate the xenobiotic genes, we wanted to know how the pathway is activated. Since during DR, fatty acid oxidation is coupled to fatty acid synthesis, we hypothesized that a certain species of PUFA may activated p38 during DR. So, we performed GC-MS and found that PUFA biosynthesis is upregulated although triglyceride levels decrease during DR. We confirmed this biochemical observation by using mutants defective in various stages of PUFA biosynthesis.

We found that the life span of *drl-2*, a serine-threonine kinase, was independent of the IIS, TORC1, AMPK and JNK signalling. Interestingly, although *drl-2* does not function in the DR pathway, it requires the DR-specific transcription factor PHA-4. We also showed that the nuclear hormone receptor NHR-8 is required for *drl-2*-mediated xenobiotic gene upregulation and longevity.

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

We studied the role of ERAD genes in DR-mediated life span regulation. We found that the life span of *eat-2(ad1116)* mutant, a genetic mimic of DR, is suppressed when the ERAD genes are knocked down using RNAi. Further, we showed that the delayed $PolyQ_{40}$ aggregation during DR is dependent on the ERAD genes. Importantly, transient supplementation of tunicamycin during development was sufficient to upregulate ERAD genes and ameliorate $PolyQ_{40}$ aggregation in an IRE-1-dependent manner.

Publication

Original peer-reviewed article

1. Maity S, Rajkumar A, Matai L, Bhat A, Ghosh A, Agam G, Kaur S, Bhatt NR, Mukhopadhyay A, Sengupta S, Chakraborty C (2016) Oxidative homeostasis regulates response to reductive Endoplasmic Reticulum stress through translation control. *Cell Rep.* **16**: 851–865.

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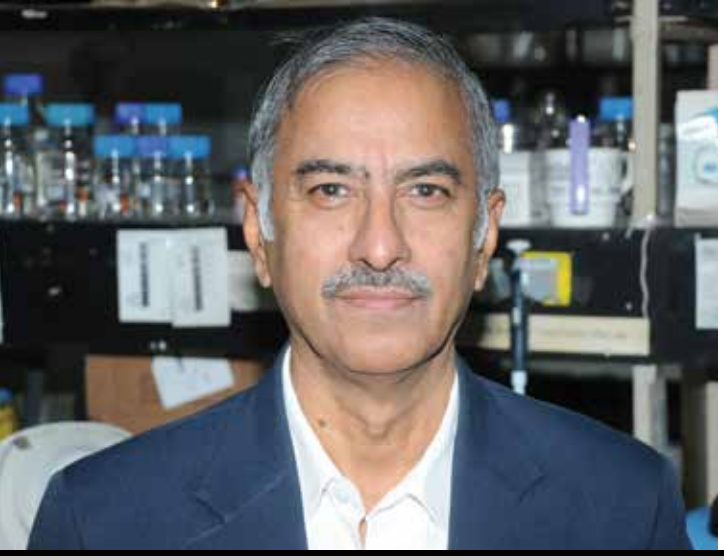

Molecular biology of infectious diseases

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Analysis of rCPB-generated host cell response:

Gram positive *Clostridium perfringens* is a major cause of human and veterinary enteric diseases largely because this bacterium can produce several toxins when present inside the gastrointestinal tract. 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 the present study, an attempt was made to understand the mechanism of action of beta-toxin and possible host cell response using *in vitro* proteomics and phosphorylation studies in CPB-susceptible THP-1 cells. Since the recombinant proteins purified from *E. coli* often have LPS contamination, LPS content of the purified rCPB was quantified. Therefore, for proteomic studies, cells treated with equivalent amount of LPS were included as control to distinguish the host cell response specific to rCPB. In order to determine host cell response against CPB, sublytic concentrations of the rCPB were used and both immediate transient as well as delayed type of responses were investigated. The immediate host cell responses results in change of phosphorylation status of the downstream signalling molecules while delayed responses involve up-regulation and down-regulation of the proteins involved in host cell response. Differential in-gel electrophoresis (DIGE) and Phospho-MAPK array for up- and down-regulation of protein synthesis and phosphorylation, respectively were employed for this analysis.

The DIGE analysis revealed down-regulation of the E181 Dj-1 protein, reported to protect cells from oxidative stress, (suggesting incapability of cells to overcome toxicity.) The rCPB treated cells showed significant reduction in KIAA0150 protein and TASP-1 (Taspase, threonine aspartase, 1, isoform CRA_a) proteins as well. Earlier microarray studies on THP-1 cells have shown overexpression of KIAA0247 (a

complement related protein), upon incubation with LPS, suggesting its possible role in oxidative stress in general which is not specific to CPB. (Taspase1 is a threonine protease responsible for cleaving intracellular substrates but full repertoires of its targets are not yet known.)

The Phospho-MAPK array revealed differential phosphorylation status of three major families of MAPK pathways- the extracellular signal-related kinases (ERK1/2), C-Jun N-terminal kinases (JNK1-3) and p38 isoforms (α/β/ γ /δ). Phosphorylation status of p38β, p38 γ, JNK2, JNK pan, Akt2, RSK1, GSK-3 α/β, MSK2 and TOR involved in cell survival under stress were found to have increased while that of CREB (cAMP response element-binding protein) to have decreased. The present study identifies Akt2, GSK-3 α/β, TOR and CREB as novel targets involved in host cell response to rCPB.

Thus, the analysis resulted in identification of the molecules involved in both immediate and delayed host response. Further studies involving deletion/manipulation of these molecules could further highlight their exact role in CPB-induced host cell response and give an insight into the pathway involved therein.

Publications

Original peer-reviewed articles

1. Dhaulakhandi DB, Ravi AK, Garg LC, Ahluwalia KB (2017) Symmetry patterning by the causative organism of Rhinosporidiosis in culture. *Romanian J Rhinology.* **7**:19-26.
2. Karan S, Dash P, Kaushik H, Sahoo PK, Garg LC, Dixit A (2016) Structural and Functional Characterization of Recombinant Interleukin-10 from Indian Major Carp. *Labeo rohita*. *J Immunol Res.* **2016**: 3962596.
3. Acharya A, Garg LC (2016) Drug Target Identification and Prioritization for Treatment of Ovine Foot Rot: An In Silico Approach. *Int J Genomics.* **2016**:7361361.
4. Mohapatra A, Karan S, Kar B, Garg LC, Dixit A, Sahoo PK (2016). Apolipoprotein A-I in Labeo rohita: Cloning and functional characterisation reveal its broad spectrum antimicrobial property, and indicate significant role during ectoparasitic infection. *Fish Shellfish Immunol*. **55**:717-728.
5. Chatrath S, Gupta VK, Dixit A and Garg LC (2016) PE_PGRS30 of Mycobacterium tuberculosis mediates suppression of proinflammatory immune response in macrophages through its PGRS and PE domains. *Microbes Infect.* **18**:536-542.

Patents

1. Garg LC, Dixit A and Keshav G (2016) A recombinant vaccine against Clostridium Perfringens infection and Epsilon (ε) toxin intoxication (*US patent no. 9408900 granted on 09/08/2016*).
2. Garg LC, Dixit A and Keshav G (2016) Recombinant non-toxic protein vaccine against Clostridium perfringens infection and epsilon toxin Intoxications (*Australian patent no. 2011275507 granted on 14/07/2016*).

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

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Regulation of nuclear processes like DNA replication, transcription, and VDJ recombination requires interactions of several cis-acting elements and trans-acting factors. Appropriate organization of chromatin at various levels is essential to accomplish spatial and temporal regulation of these vital processes which impact metazoan development and cellular functions. CTCF has emerged as an important contributor to chromatin organization. The details of its ability to organize chromatin are confounded by the multifunctional attributes of CTCF and diversity in its functions. To understand these aspects, we are investigating the role of CTCF in regulation of transcription and VDJ recombination at TCRb locus.

Antigen receptor loci like IgH, TCRa/d, TCRb etc. present a useful framework to explore the nature of interactions amongst various regulatory elements. At these loci, transcription as well as RAG mediated VDJ recombination is exquisitely regulated during development. 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 gene segments. These segments are located at large distances from each other on the chromosome and higher order chromatin reorganization is necessary to bring them together prior to recombination. Since CTCF is an important global factor contributing to long range interactions of chromatin, it is of interest to decipher the chromatin structure and organization of the wild type and genetically manipulated TCRb loci to understand various aspects of CTCF based chromatin organization that may influence the interactions of regulatory elements important for transcription and VDJ recombination.

While the importance of CTCF in chromatin organization is clear, there is considerable genome-wide diversity in the occupancy of CBS by CTCF, degeneracy in the nucleotide

sequence of CBS, variations in the chromatin state of CBS, variations in the protein partners that collaborate with CBS and/or might bind the flanking regions to stabilize or destabilize CTCF binding. Adding to this variation, location of the CBS in context of other regulatory elements, also impacts its function. Not surprisingly, the functional outcomes of CTCF binding are diverse. Also, there is only limited understanding regarding the mechanisms that lead to CTCF-mediated chromatin domain organization. Recent observations that orientations of CBS play an important role in this organization have led to the proposal that extrusion of chromatin and its curtailment by CTCF/cohesin in a CBS orientation dependent manner is critically important for organization of chromatin domains relevant for transcription. More locus specific analysis is essential to further understand the CTCF mediated chromatin organization and function.

TCRb has 21 CTCF binding sites (CBS) identified by ChIP-seq. CBS are in the proximity of the V segments and also flank the Recombination Center (RC) and exhibit interactions amongst themselves in a complex manner as discerned by Chromosome Conformation Capture (3C-qPCR) assay. Our analysis in previous years has revealed that multiple CBS at TCRb locus exhibit diversity in the CBS occupancy by CTCF as well as the intrachromosomal interactions mediated by them. Based on the CBS diversity and/or their location, CBS are likely to have distinct roles in regulating transcription and VDJ recombination at TCRb.

At TCRb locus, the location of CBS sites and their interactions suggest that they could be

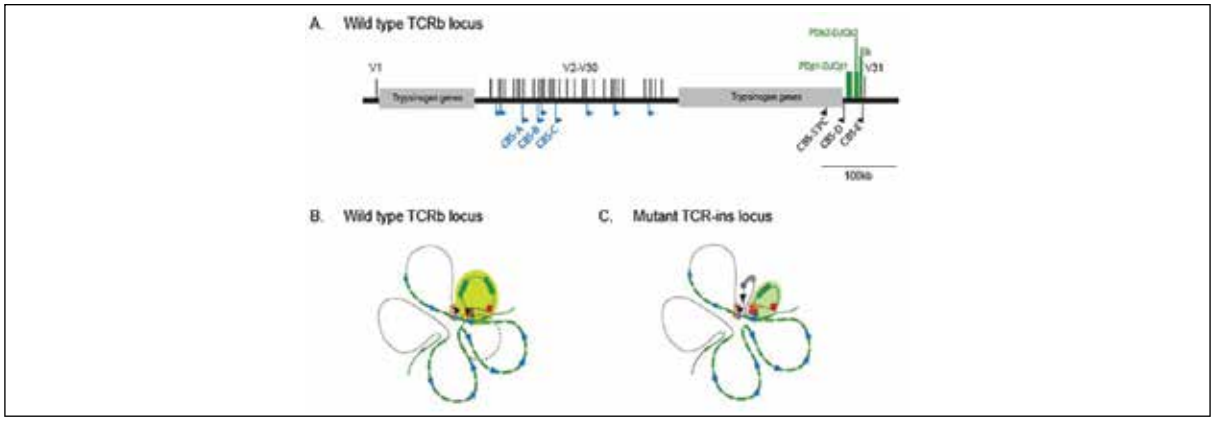

Figure 1. Model to depict chromatin loop configuration of TCRb locus that might arise by chromatin extrusion in wild type and TCR-ins mutant loci. A. Linear map of TCRb locus showing relative positions of RC, V segments and a subset of endogenous CBS of TCRb locus. **B.** Two dimensional depiction of the chromatin loop configuration of the wild type locus. From the ensemble of possible combinations that can organize the CTCF dependent loops, one conformation is compared wherein two extrusion complexes have been considered. CBS-D and CBS-E (black arrowheads) based chromatin loop encompasses Eb (red circle) and promoters PDb1 and PDb2 (green circles) each linked to a DJC cluster (green rectangles). Eb based activation of this domain creates the RC (yellow green circle) leading to D-to-J recombination. The second extrusion complex involves CBS-5'PC and any one of the CBS (blue arrowheads) located in the domain (green lines) encompassing V gene segments. Consequently, depending on the CBS that halts the extrusion, some V segments are extruded away while a few are brought to the vicinity of the RC. Flexibility of the chromatin loop of RC (depicted as black dashed circle), as well as of the chromatin loop encompassing the V segments; allow aligning of RSS elements necessary for V-to-DJ recombination. Extrusion of V segments proximal to RC is likely to favour the utilization of distal V segments. Domains encompassing inert Trypsinogen genes are shown as grey lines. **C**. Two dimensional depiction of the chromatin loop configuration as altered in TCR-ins. Due to interaction of CBS-ecto (red triangles) with CBS-E, the Eb activated RC is smaller as well as oriented differently (shaded green oval) and /or is less flexible. PDb1-DJCb1 region (grey circles and rectangle) get excluded from the RC. The other extrusion process that brings V to RC proximity may or may not be affected depending on the dynamics of extrusion. However, due to the altered orientation and/or flexibility of the RC, the upstream V segments, despite reasonable proximity to the RC, are not able to align appropriately for V-to-DJ recombination. Spatial proximity of V31 (a green dash downstream to Eb) does not change and being suitably aligned, it is used for V-to-DJ recombination in wild type TCRb locus as well as TCR-ins. In each case, loops are not in proportion to the linear span of DNA and for clarity RC is shown to be significantly larger. Also, additional proteins that may stabilize some of the looped configurations are not depicted as they have not yet been identified.

important for maintaining the integrity of RC and/or facilitating locus contraction that precedes V-to-DJ recombination in Double Negative (DN) thymocytes. Our analyses of wild type and mutant TCRb loci suggest that perturbations in CTCF dependent interactions are reflected in the loss of functional integrity of RC as well as alterations in the interactions of the RC to the V segments leading to alterations in choice of V segments for V-to-DJ recombination. In addition to proximity, the precise relative configuration of chromatin loop encompassing RC and the V segments emerged to be important for RSS mediated recombination and could be influenced by the CBS interactions.

Besides revealing the importance of CTCF mediated chromatin organization for TCRb regulation, the observed chromatin loops in wild type and TCR-ins mutant loci were consistent with the emerging idea that CBS orientations influence chromatin loop organization. In this context, it is interesting to examine the intrachromosomal interactions at TCRb locus (Figure 1) keeping in view the tenets of chromatin extrusion model for demarcation of chromatin domains. While importance of CBS orientations has been emphasized for creation of domains critical for transcriptional regulation, our analysis also underscored the importance of CBS orientations for defining chromatin architecture that supports VDJ recombination.

Our observations have significant implications beyond the regulation of AgR loci as they indicate that even mild changes in the CTCF based long-range chromatin architecture as well as localized chromatin folding, which may arise due to enhanced or lowered CTCF binding at specific CBS for a variety of reasons, can have profound functional consequences for gene expression. This is particularly relevant for developmental decisions as well as cellular functions as CTCF based chromatin architecture is important for transcriptional regulation of several genes in metazoan genomes. To understand these interdependent aspects pertinent for generation of chromatin domains and regulation of transcription and VDJ recombination, we have initiated several experiments that are ongoing.

Publications

Original peer-reviewed articles

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2. Kochat V, Equbal Z, Baligar P, Kumar V, Srivastava M, Mukhopadhyay A (2017) JMJD3 aids in reprogramming of bone marrow progenitor cells to hepatic phenotype through epigenetic activation of hepatic transcription factors. *PLoS One* **12**:e0173977.

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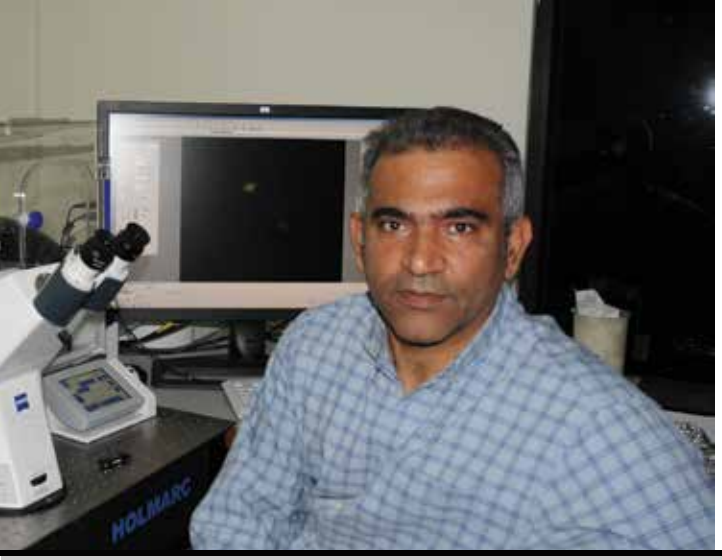

Role of cell signaling in eukaryotic development

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We are interested in 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.

We have deciphered a role for PfCDPK1 in host RBC invasion. Several novel substrates for PfCDPK1 were identified using a quantitative proteomics approach and several of its targets were validated. Previous studies in the laboratory hinted that PfCDPK1 may cross-talk with cAMP signalling. We demonstrated that the regulatory subunit of cAMP-dependent Protein Kinase (PfPKA-R) may be phosphorylated by PfCDPK1 in the parasite at S149. In addition, the association of PfPKA-R with PfCDPK1 was also established in the parasite. PfCDPK1 depletion resulted in an enhanced association between PfPKA-R and PfPKA-C. Concomitantly, a decrease in PfPKA-C activity in the parasite was observed. On the basis of these studies, we concluded that PfCDPK1 may facilitate the dissociation of PfPKA-R with PfPKA-C, which may be important for PfPKA activation. Further studies, indicated that the cross-talk between PfCDPK1 and PfPKA may be important for RBC invasion.

b. Role of phosphoinositides in parasite signaling and trafficking

Apicomplexan parasites like *Plasmodium falciparum* and *Toxoplasma gondii*, which are severe human disease causing pathogens, possess only a subset of autophagy related proteins (ATG) and their functions have remained largely unknown. One of the major effectors of PI3-Kinases in most organisms is autophagy related gene ATG18 or its homologues like WIPI1 (WD Repeat Domain, Phosphoinositide Interacting 1), which interact with 3'-phosphorylated

phosphoinositides (3'-PIPs). We identified a homologue of ATG18 in *P. falciparum* and *Toxoplasma gondii*. Pf/TgATG18 interacted with 3'PIPs *via* a conserved FRRG motif. The depletion of parasitic PI3P lead to retention of Pf/TgATG18 in the cytoplasm. Furthermore, mutations of the FRRG motif lead to a loss in vesicular localization of Pf/TgATG18. These data suggested that 3'PIPs- regulate cellular trafficking of Pf/TgATG18 in parasites.

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

The reactivation of the cell cycle in response to neurotoxic insults leads to neuronal cell death and some cell cycle related proteins contribute to this process. p27 kip1 (p27), an inhibitor of Cyclin Dependent Kinases (CDKs), prevents unwarranted CDK activation. We elucidated a novel mechanism *via* which p27 promotes apoptosis of neurons stimulated by neurotoxic amyloid peptide $A\beta_{42.}$ Co-immunoprecipitation revealed that p27 promotes interaction between Cdk5 and Cyclin D1, which was induced by $A\beta_{42}$ in cortical neurons. As a result, Cdk5 was sequestered from its neuronal activator p35 resulting in kinase deactivation. The depletion of p27 restored Cdk5-p35 interaction by preventing the association between Cdk5 and Cyclin D1 and also abrogated $A\beta_{42}$ induced apoptosis of cortical neurons. Furthermore, analysis of cell cycle markers suggested that p27 may play a role in $A\beta_{42}$ induced aberrant cell cycle progression of neurons, which may result in apoptosis. These findings provided novel insights into how p27-which otherwise performs important neuronal functions- may become deleterious to neurons under neurotoxic conditions.

Publications

Original peer-reviewed article

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DOI: 10.1002/9781118493816.ch10

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

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BLM recruitment to annotated double-standed breaks (DSBs) in the genome is independent of its helicase activity

In an effort to understand the mechanism of BLM recruitment at the DSBs, we carried out a time-dependent 4-OHT treatment in U2OS AsiSI ER cells in which the restriction enzyme AsiSI is stably integrated in the genome. Treatment with 4-OHT leads to translocation of AiSI restriction enzyme to the nucleus, which causes cleavage of the genomic DNA at annotated sites and formation of a DSB. BLM was induced concomitantly with γH2AX, NBS1 and autophosphorylated ATM. Consequently BLM colocalized with these proteins at 4 hrs after 4-OHT treatment.

To elucidate whether BLM was recruited to the exact annotated DSBs, chromatin immuno-precipitation (ChIP) experiments were carried out in U2OS-AsiSI-ER cells, grown either in asynchronous conditions or after 4-OHT treatment for 4 hrs. ChIP-qPCR was carried out on ten DSBs spread across eight chromosomes. BLM recruitment occurred only after the induction of DSBs due to 4-OHT treatment at all the tested DSBs. The recruitment extended from approximately 100bp to 10 kb in a biphasic manner. BLM ChIP was also carried out in presence of ML216, which acts as a specific inhibitor for BLM helicase activity by competing with DNA for binding to BLM. Presence of ML216 did not prevent BLM from being recruited to the DSBs.

MRN complex and ATM control BLM recruitment at the DSBs

Reciprocal immunoprecipitations between BLM and MRN members demonstrated that BLM interacted with MRN complex – the interaction being enhanced after damage induction. Next we wanted to determine whether BLM recruitment at the DSBs depended on MRE11 and/or ATM activity. After treatment with either KU 55933 (ATM kinase inhibitor) or Mirin(MRE11 exonuclease activity inhibitor) the recruitment of BLM to either proximal or distal region of multiple DSBs was found to be unaffected in absence of ATM activity. However in presence of Mirin, lesser amount of BLM was recruited to all the DSBs. These indicated that MRE11 exonuclease activity but not ATM was partially required for BLM recruitment at the

DSBs.To determine whether the protein levels of ATM and MRE11 are directly required for BLM retention at the DSBs, BLM ChIP was carried out in U2OS-AsiSI-ER cells after depleting NBS1, MRE11 or ATM. Lack of any of these factors did not allow BLM to be recruited.

BLM negatively regulates HR and c-NHEJ

Next we wanted to determine whether BLM recruitment to the DSBs in different phases of the cell cycle allowed it to affect the two main repair pathways – HR and c-NHEJ. ChIPs were carried out in untreated or 4-OHT treated cells synchronized either in S or G1 phase of the cell cycle by double thymidine block and subsequent release for different time intervals. During the S-phase, BLM and RAD51 (and very little XRCC4) were recruited to both the proximal and distal regions of all the tested DSBs after 4-OHT treatment. Interestingly, BLM was also recruited during G1 phase to the proximal and distal regions of all the DSBs along with XRCC4 but not RAD51. Hence it was hypothesized that BLM will preferentially interact with HR and c-NHEJ proteins in S- and G1 phases, respectively.

In order to determine whether BLM could specifically regulate HR and c-NHEJ in a cell cycle specific manner, HR and c-NHEJ assays were carried out with extracts prepared from cells arrested in S- and G1-phase. The cells were either left untreated (-4-OHT) or treated with 4-OHT alone or 4-OHT in presence of ML216, B02 (a RAD51 inhibitor which disrupts the binding of RAD51 to DNA) or SCR7 (a Ligase IV inhibitor which blocks Ligase IV mediated end joining by interfering with the DNA binding of Ligase IV). As expected, lack of pro-recombinogenic protein, RAD51, decreased the rate of HR to the basal level during S-phase. Inhibition of BLM activity by ML216 treatment caused an increase in the rate of HR. This negative effect of BLM on HR was only during S- and not in G1-phase, essentially due to the lack of sister chromatids in the latter phase. In G1 phase, BLM negatively affected c-NHEJ. Hence lack of functional BLM due to ML216 treatment, increased the rate of joined product formation. As expected, treatment of cells with SCR7 abolished c-NHEJ, while the treatment with B02 forced the cells in S-phase to forgo HR and instead adapt c-NHEJ as the repair pathway. Interestingly, treatment of ML216 increased c-NHEJ in S-phase. This indicated that though HR is the pathway preferentially inhibited by BLM, it (i.e. BLM) also exerts its negative regulatory effect on c-NHEJ during S-phase.

Publication

Original peer-reviewed article

1. Kumari J, Hussain M, De S, Chandra S, Modi P, Tikoo S, Singh A, Sagar C, Sepuri NB, Sengupta S (2016) Mitochondrial functions of RECQL4 are required for the prevention of aerobic glycolysis-dependent cell invasion. *J Cell Sci.* **129**:1312-1318.

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Understanding the regulation of DNA replication

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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 investigating the cellular response to aberrations in replication complexes. The objective is to identify yet unknown checkpoint pathways that monitor the replication apparatus. We are evaluating the role of microRNA in regulating the DNA replication machinery as the cell progresses from one phase to the next. 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.

GINS4 is required for centrosome integrity during mitosis

We report that GINS4, also known as Sld5 is essential for resisting the forces that converge on centrosomes during chromosome congression. Since unaligned chromosomes were the first aberrations to appear after Sld5 depletion, we wanted to examine the role of these forces in spindle pole fragmentation. When Eg5 kinesin was inhibited after Sld5 depletion by incubation with monastrol, we observed that cells formed monopolar short spindles with closely spaced centrosomes, indicating that spindle pole fragmentation occurs after centrosome separation in Sld5-depleted cells (Figure 1). We observed that co-depletion of CENP-E suppressed the spindle pole aberrations caused by Sld5 depletion,

though some congression defects are observed (Figure 2). This indicates that depletion of Sld5, per se, did not lead to loss of spindle pole integrity,which resulted due to the inability of Sld5-depleted centrosomes to withstand the CENP-E-mediated forces.

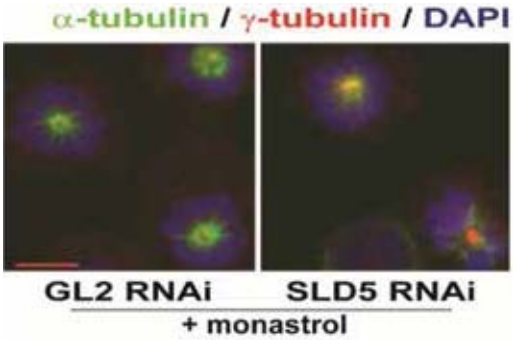

Figure 1. Spindle pole defects observed after monastrol treatment demonstrate that centriole splitting occurs after centrosome separation at prophase. Merged images of control *GL2* or *SLD5* siRNA transfected cells co-stained for α-tubulin (green), γ-tubulin (red), and DNA by DAPI (blue) after incubation with monastrol for 4 h. The scale bar is 10 μm.

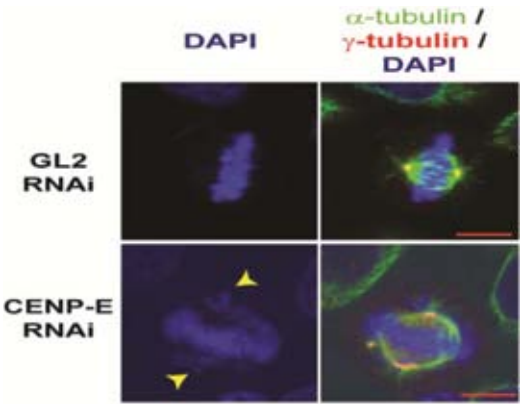

Figure 2. Inhibition of CENP-E results in multiple chromosome congression defects. HeLa cells transfected with control *GL2* or *CENP-E* siRNA were co-stained for α-tubulin (green) and γ-tubulin (red) while TOTO-3 was used to stain the nucleus (pseudo-colour blue). Arrowheads point to unaligned chromosomes observed in CENP-E depleted samples. The scale bar is 10 μm.

Role of non-coding RNAs in the regulation of cell cycle

We report that a microRNA, miR-874 downregulates the major G1/S phase cyclin, CCNE1 during serum starvation to enable cell survival. Inhibition of miR-874 activity during nutrient-deprived conditions results in reduced cell viability. The 3' untranslated region (UTR) of CCNE1 gene harbors a binding site for miR-874, which is essential for repression by miR-874. Mutation of this recognition element results in abrogation of repression. In nutrient-sufficient conditions, miR-874 is downregulated resulting in CCNE1 prevalence and cell growth. The regulation of miR-874 is apparent in the context of oncogenesis: In aggressive osteosarcomas, miR-874 is downregulated leading to an upregulation of CCNE1, whereas its restoration suppresses aggressive growth phenotypes, such as migration, invasion, and cell survival. The delivery of miR-874 to tumor cells resulted in marked regression of xenografts in nude mice demonstrating an anti-proliferative activity of miR-874. In summation, we report that miR-874 ensures cancer cell survival by limiting proliferation in nutrient- deprived conditions, and is downregulated during aggressive growth stages.

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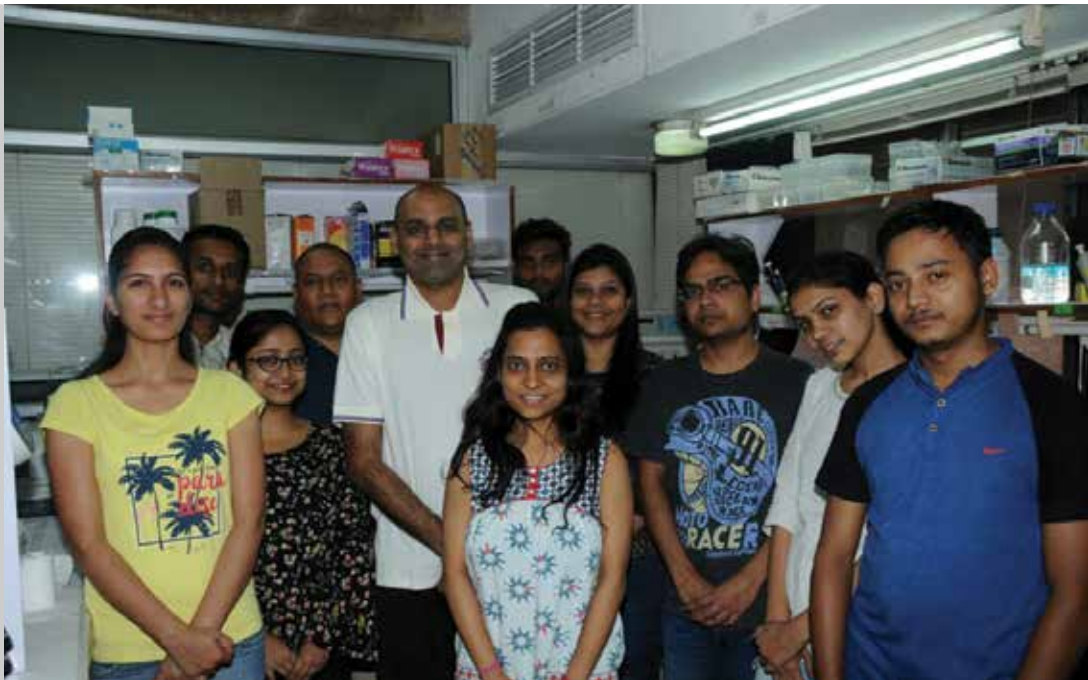

The role of tumor suppressors in stress response

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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 two such proteins viz. sirtuin 6 (SIRT6) and cyclin F. At the molecular level, SIRT6 regulates the expression of a large number of stress-responsive and metabolism related genes. Since SIRT6-deficient mice exhibit profound metabolic defects understanding the role of SIRT6 in metabolic homeostasis is an area of notable interest. Cyclin F is the substrate recognition subunit of SCF (Skp1-Cul1-F-box protein) ubiquitin ligase complex. We plan to investigate the role of SCF ubiquitin ligase complex in regulating metabolic processes.

UBE3A is an E3 ubiquitin ligase well known for its role in p53 proteasomal degradation in HPV (human papillomavirus)-associated cancers. However, apart from p53 degradation, the role of UBE3A E3 ligase activity in tumorigenesis is poorly understood. Here we report that UBE3A ubiquitylates sirtuin 6 (SIRT6) leading to its proteasomal degradation. We further mapped the site of ubiquitylation to the highly conserved Lys160 residue in SIRT6. FOXO1-mediated transcriptional repression of UBE3A promotes SIRT6 stabilisation. We further show that SIRT6 represses ANXA2, a key mediator of UBE3A oncogenic functions, through an epigenetic mechanism. Thus UBE3A-mediated SIRT6 degradation promotes proliferation capacity, migration potential and invasiveness of cells. Additionally, our studies in mouse tumor models demonstrate the critical role of SIRT6 downregulation and consequent induction of ANXA2 in UBE3A-mediated tumorigenesis. Furthermore, increased UBE3A levels correlate with reduced SIRT6 levels and elevated ANXA2 levels in increasing grades of hepatocellular carcinoma. These findings provide new insights into the regulation of SIRT6 and establish the molecular mechanism underlying UBE3A-mediated tumorigenesis.

Previously, we had performed a gene expression microarray analysis to identify genes regulated by metabolic stress response. Cyclin F was among the genes robustly induced upon metabolic stress. In order to examine the functions of cyclin F under metabolic stress conditions, we sought to identify its interacting partners. Among the novel interactors RBPJ (Recombination Signal Binding Protein for Immunoglobulin Kappa J Region) was of particular interest as it is an effector of the Notch signaling pathway. It functions both as a repressor as well as a transcription factor. A number of genes that are involved in various cellular processes that govern tumorigenesis are shown to be regulated by the RBPJ-Notch pathway. But not much is known about the role of notch signaling pathway in determining cellular metabolic outcome. Since cyclin F induced in response to metabolic stress, we decided to investigate its role in regulating notch pathway in cellular metabolic processes which may have broader implications for tumorigenesis.

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

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The laboratory aims at advancing our molecular understanding of skeletal growth by studying the functions of molecules of skeletal or extra-skeletal origin in the regulation of bone mass. Two distinct programs of work are being pursued in the laboratory at the moment. First one is investigating how taurine regulates osteoblast functions and whether manipulation in its levels during growth or ageing has therapeutic implications (A). In the second program of work we are investigating how pineal-derived melatonin regulates bone mass through its receptors expressed on the bone cells (B).

A. Taurine regulation of bone mass and its therapeutic implications:

The aim of this project is to investigate the molecular basis of taurine action in osteoblasts and to investigate the therapeutic relevance of the taurine-bone axis in treating low bone mass disorders. In the last one year, we have gathered multiple lines of evidence to show that:

1. Taurine increases bone mass during growth: In the first set of experiments we investigated whether daily oral administration of taurine increases bone mass in growing mice. Bone histomorphometric analysis of the skeleton showed that mice that received taurine at either 500 mg/kg/day and 1000 mg/kg/day orally increased bone mass **(Fig 1 A)**.

2. Taurine prevents bone loss following gonadal failure:
To address whether taurine administration has the potential to prevent bone loss following menopause, mice were either ovariectomized or sham operated and treated with either vehicle or taurine daily for 6 weeks. Results revealed that daily treatment with taurine at 500 or 1000 mg/kg per day dose was sufficient to prevent bone loss caused by gonadal failure **(Fig 1B)**.

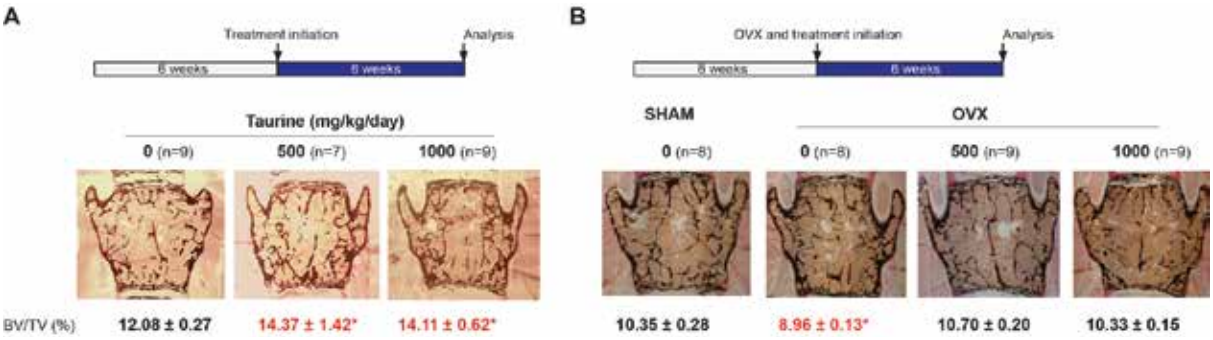

Figure 1. Taurine increases bone mass during growth and prevents osteoprosis following gonadal failure
(A) Taurine treatment for 6 weeks following gonadal maturation increases bone mass. Representative image of VonKossa stained vertebra from female mice gavaged with either 0, 500 or 1000 mg/kgBW/day of taurine for 6 weeks. (B) Taurine treatment following gonadal failure increases bone mass. Representative images of VonKossa stained vertebra from mice that were either sham operated or ovariectomized at 6 weeks of age and gavaged daily with either 0, 500 or 1000 mg/kgBW/day of taurine in a preventive mode for 6 weeks. *P<o.05; n in each group is indicated in brackets.

B. Melatonin regulation of bone mass and its therapeutic implications:

Melatonin is a tryptophan derivative synthesized primarily by the pineal gland in the brain that acts on different tissues of the body to regulate their functions through two known cell surface receptors, Mtnr1a and Mtnr1b. We have recently accumulated preliminary evidence using mouse genetic and *ex vivo* models that pineal-derived melatonin (PDM) regulates bone mass. Loss of function of melatonin synthesis specifically in the pineal gland, through the use of *Tph1* floxed mice and a pineal-specific *Cre* driver (*Crx-Cre*) (Fig. 2A), leads to a low bone mass phenotype due to a decrease in osteoblast numbers and bone formation rate without any effect on bone resorption. Analysis of mouse genetic models of receptor deficiency showed that melatonin in adult mice regulates bone mass by acting through the Mtnr1b and not the Mtnr1a receptors (Fig. 2B). We further showed that one mechanism through which Mtnr1b regulates osteoblasts is through cell autonomous decrease in osteoblast functions

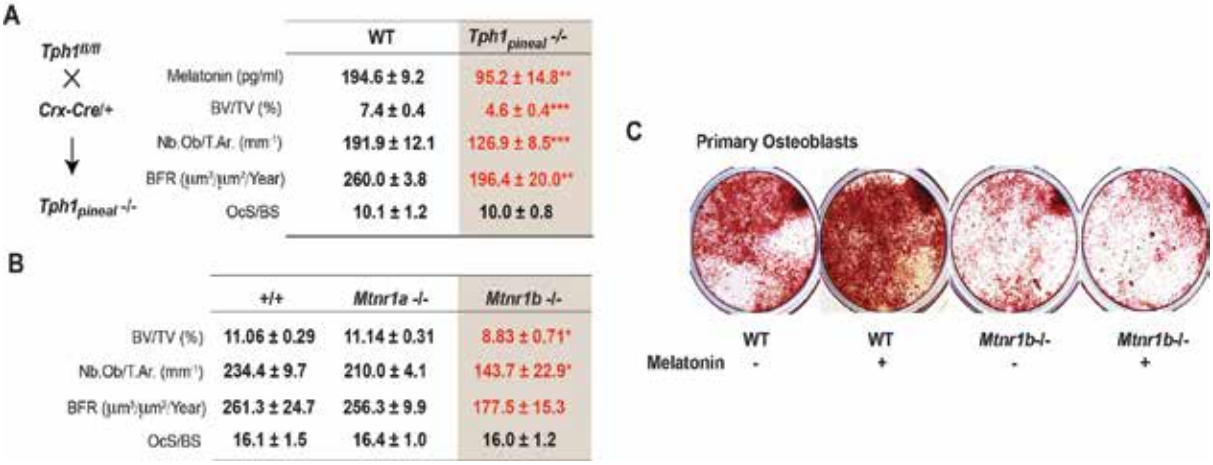

Figure 2. Pineal-derived melatonin positively regulates bone mass
(A-B) Schematic representation of the breeding strategy to obtain $Tph1_{pineal}$-/- and histological analysis of vertebra in 12 week-old wild-type and $Tph1_{pineal}$ -/- mice (A) and wild-type, *Mtnr1b-/-* and *Mtnr1b-/-* mice (B). (C) Osteoblast mineralization assay in WT or *Mtnr1b-/-* osteoblasts treated with veh/melatonin for 21 days through alizarin red staining *P<0.05; **P<0.01; ***P<0.001

and that these cells are refractory to exogenous melatonin addition (Fig. 2C).

From a biomedical point of view the emergence of melatonin as a potent activator of bone formation raises the prospect that manipulation of its levels/ action could be a novel means to treat low bone mass disorders.

Left to Right
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Role of non-coding RNA mediated gene regulation in human development and disease

G. Aneeshkumar Arimbasseri

Rare genetic disease diagnosis is devastating for families; most of them have no treatment options. Understanding the biological mechanisms of rare diseases is essential for finding cures and for developing better management strategies. Our laboratory addresses a class of rare genetic diseases: tRNA and RNA Polymerase III associated genetic disorders.

tRNAs are short non-coding RNAs that are used as adapters for protein synthesis. Their function is to match the codons with cognate amino acids. In recent years, many genetic disorders have been identified to be associated with mutations that affect tRNA biogenesis pathways. Our aim is to understand the basic principles that connect these mutations to the diseases.

Role for tRNAs are also implicated in infectious diseases, especially infection by persistent viruses such as HIV. These viruses alter the relative composition of tRNAs (tRNA pool) in the host cells upon infection to aid better translation of the viral mRNA. It has been shown previously that the natural mechanisms for host cells to resist viral infections involve blocking the tRNA pool modulation by these viruses. Thus, the tRNA pool modulation may provide novel drug targets for viral infections.

Major projects underway in our laboratory are:

A) Role of tRNA biogenesis in neurodevelopment.

Mutations in several tRNA modification enzymes are known to be associated with neurodevelopmental disorders. Many of them including the mutations in TRMT10A, that is responsible for 1-methylguanosine at position 9 (m1G9) of a subset of tRNAs; and WDR4, which is responsible for 7-methylguanosine at position 46 (m7G46) tRNAs, cause microcephaly. We plan to generate the cerebral organoid models for these mutations to find out defects in gene expression caused by these mutations that lead to microcephaly.

B) Role of RNA Polymerase III transcribed non-coding RNAs in myelination.

Many patients from across the world with hypomyelinating leukodystrophy have recently been identified to carry pathogenic mutations in catalytic subunits of RNAP III. The mechanism by which the mutations in RNAP III lead to such a devastating disease is unknown. Currently, we aim to generate cerebral organoid models carrying the patient mutations to understand the mechanism by which these mutations translate in to diseases.

C) Mechanism of tRNA pool modulation by HIV.

Genes of persistent viruses such as HIV exhibit a codon usage significantly different from the host codon usage. This difference in the codon usage should put HIV gene expression at a disadvantage; as evidenced by attempts to express HIV late genes in human cells under a constitutive promoter. But, upon infection, HIV genes appears to be translated efficiently. Some evidence shows that during infection, HIV alters the tRNA pool of the cells to match the codon usage of HIV genes. It has also been shown that host cells resist HIV infection by

preventing tRNA pool modulation by HIV. We attempt to identify the mechanism of tRNA pool modulation by HIV. Understanding this mechanism is expected to have implications in designing novel anti-viral therapies.

Publication

Original peer-reviewed article

1. Maraia RJ, Arimbasseri AG (2017) Factors that shape eukaryotic transcriptomes: processing, modification and anticodon usage. *Biomolecules.* **7**: 26

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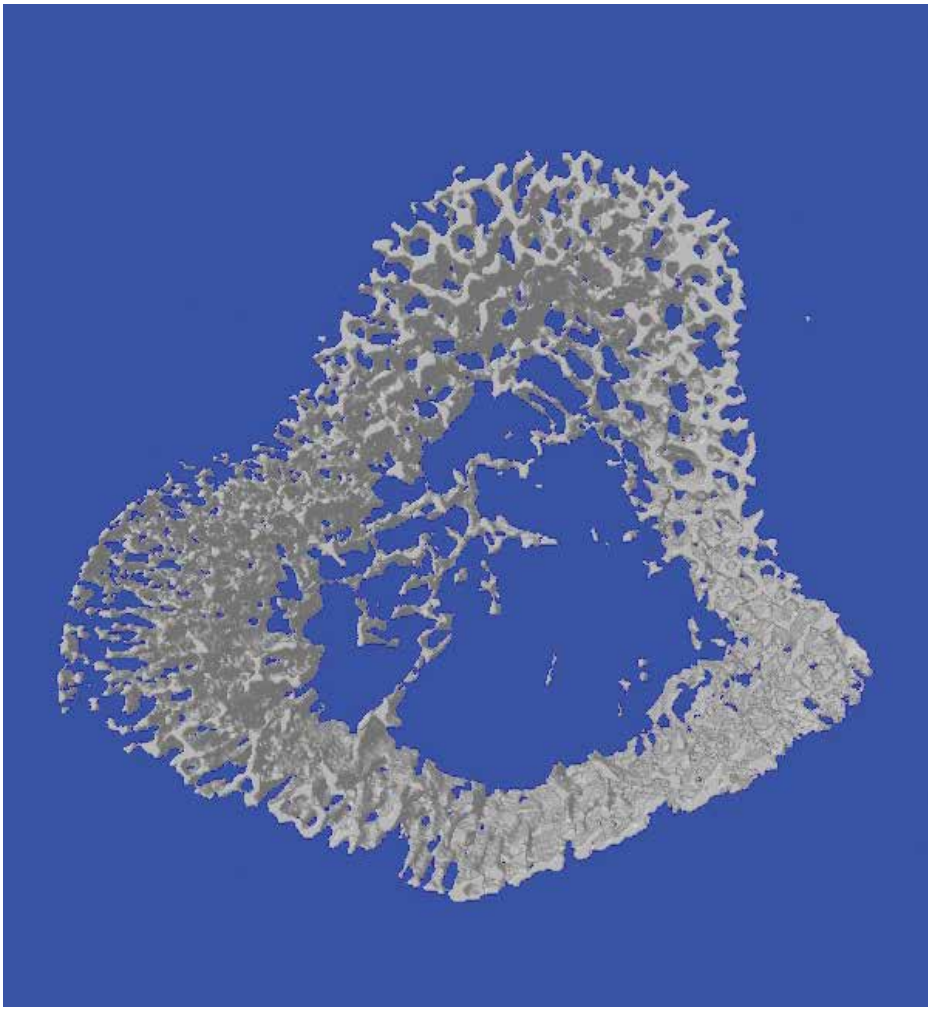
Anamica Das
Khim Singh



Micro-computed tomography image from mouse long bone (tibia) shows the central woven and peripheral compact bone architecture.

ANCILLARY RESEARCH

Production of transgenic and other animal models for biomedical research

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Theme of the research is to generate transgenic animals for the study of functional genomics and mammalian development and other animal models for use in biomedical research.

- To develop transgenic animal using genes relevant to human health and diseases as well as to use this technology for making animals expressing therapeutic products in milk for increasing affordability of such therapeutics.
- To study biology of spermatogonial stem cells and use germ cell transplantation for restoration of fertility following chemotherapy.
- To provide services of making various transgenic animals to various laboratories of the nation.

Generation of various transgenic mice for other investigators

This service is provided by NII to various laboratories of the nation. Collaborative work for making various transgenic animals for other investigators was undertaken as and when the constructs were provided. Fore-founder animals were given to the investigator for generating transgenic lines to address their respective scientific goals.

To identify and overexpress a gene for enhanced spermatogonial stem cell self renewal and germ cell proliferation leading to efficient colonization post-transplantation.

We established a method for depletion of germ cells by injection of busulfan directly in

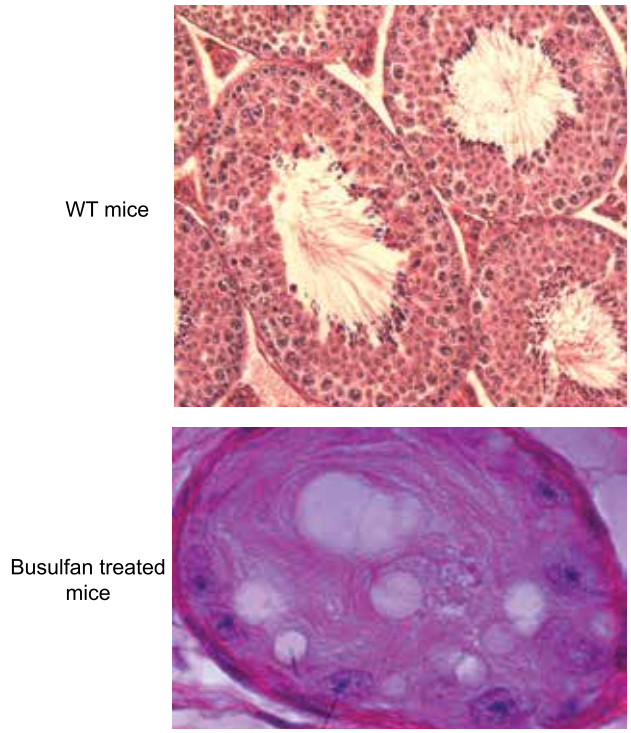

Figure: - Hematoxylin and eosin stained sections of testis after 15 days post testicular busulfan injection (75µg) in comparison to WT mice. Maximum depletion of Gc leaving behind only Sertoli cells was observed. Black arrow head marks the nucleus of Sertoli cells, red arrowhead marks the cytoplasm of Sertoli cells which was intact in busulfan treated mice.

the testis followed by efficient transfer and colonization of transplanted spermatogonial stem cells (SSC) expressing EGFP. Such transplanted mice generated normal litter. This generates hope for restoration of fertility in individuals treated with chemotherapeutic agents.

Next, we identified and overexpressed TSPAN8 gene specifically in the Sertoli cells of the mice to increase self-renewal function and proliferation of SSC so as to obtain more cells from small biopsy of testis, for efficient colonization post-transplantation. The knowledge generated in this study may offer significant translational benefits for treating male infertility in pediatric oncological patients who suffer from the gonadotoxic side-effects of radiation and chemotherapy.

Work with other animal models

Because of several problems associated with insulin injection, orally active insulin mimetic compounds would be an ideal substitute. A small molecule, a peroxyvanadate compound i.e. DmpzH[VO(O_2)$_2$(dmpz)], henceforth referred as dmp was made by Prof Mihir K. Chowdhury of Tezpur University. Prof Samir Bhattacharya showed that oral administration of dmp to streptozotocin treated BALB/c mice lowers blood glucose level and stimulates glucose and fatty acid uptake by skeletal muscle and adipose tissue respectively. In *db/db* mice (work done at NII), it greatly improved insulin sensitivity through excess expression of PPARγ and its target genes i.e. adiponectin, CD36 and aP2. Study on the underlying mechanism demonstrated increased production of adiponectin lowers circulatory TG and FFA levels, activates AMPK in skeletal muscle and this stimulates mitochondrial biogenesis and bioenergetics. The results obtained strongly indicated that dmp could be a potential candidate for insulin replacement therapy.

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H

PUBLICATIONS, PATENTS AND TECHNOLOGY TRANSFER

A. ORIGINAL PEER-REVIEWED ARTICLES

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B. REVIEW/PROCEEDINGS

1. Khater S, Anand S, Mohanty D (2016) In silico methods for linking genes and secondary metabolites: The way forward. *Synthetic and Systems Biotechnology* **1**:80-88. In Special Issue on "Bioinformatics tools and approaches for Synthetic Biology of natural products" (Eds. Tilmann Weber, Technical University of Denmark, Denmark).

2. Malhotra SS, Banerjee P, Gupta SK (2016) Regulation of trophoblast

differentiation during embryo implation and placentation: Implication in pregnancy complications. *J Reprod Health Medicin*e **2**:S26-S36.

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4. Mathur R, Shaha C (2016) Cell death profile of a protozoan parasite. In Recent Advances in Communicable and Non-communicable diseases. (Eds. A. Dutta and V.P. Sharma, NASI- Capital Publishing. *New Delhi) pp*. **244**-255.

5. Mishra A, Upadhyay PK, Nagaragan P (2016) Immunotherapy in Liver Diseases: A balance between Immunity and tolerance. *Curr Drug Metab*. **17**: 997 – 1005.

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PATENTS APPLICATION/GRANTED AND TECHNOLOGY TRANSFER

1. Garg LC, Dixit A and Keshav G (2016) A recombinant vaccine against Clostridium Perfringens infection and Epsilon (ε) toxin intoxication *(US patent no. 9408900 granted on 09/08/2016)*.
2. Garg LC, Dixit A and Keshav G (2016) Recombinant non-toxic protein vaccine against Clostridium perfringens infection and epsilon toxin Intoxications *(Australian patent no. 2011275507 granted on 14/07/2016)*.
3. Panda AK, Kanchan V (2016) Polymer particles based vaccine (A vaccine composition comprising polymeric nanoparticles and microparticles for inducing TH1 and TH2 response *(Indian patent no. IN278769 granted on 30/12/2016)*.
4. Sehgal D and Rohatgi S (2017) Immunoglobulin genes specific oligonucleotides and uses thereof. *(Indian patent no. IN280165 granted on 13/02/2017)*.
5. Polachira SK, Nair R, Jayalekha R, Gupta SK, Mishra NN and Agarwal A (2017) Herbal microbicide formulation for preventing HIV *(Indian patent application no. 201741004017 filed on 3/02/2017)*.
6. Biswal BK, Kumar D and Jha B (2017) Inhibitiors of Imidazoleglycerol-phosphate dehydratase (HISB) in the treatment of tuberculosis *(Indian patent application no. 201711011584 filed on 31/03/2017)*.

Technology Transferred

A technology – "Process for obtaining bioactive protein from inclusion bodies" has been transferred to EPR Centre for Cancer Research and Bioinformatics Private Limited (A Vitane Group of Company) located in Shamirpet Mandal, RR Dist-500078, Telangana.

AWARDS AND DISTINCTIONS

Dr. Anil K. Suri
- Prof. S. S. Guraya Memorial Award 2017 conferred by Indian Society for the Study of Reproduction and Fertility for Cancer Immunotherapy
- Elected Vice President of Indian Society for the Study of Reproduction and Fertility.

Dr. Chandrima Shaha
- Elected as Vice-President International Affairs, INSA, 2016.
- Nominated as Sectional President of the Biological Sciences Section of the NASI, 2017.

Dr. Satish K. Gupta
- Awarded The Chandrakala Hora Memorial Award, INSA.
- Received Haryana Vigyan Ratna Award (2013-14) by Haryana State Council for Science and Technology, Government of Haryana (announced in 2017).
- Elected President of the International Society for Immunology of Reproduction (2016- 2019).

Dr. Rajendra P. Roy
- Received J C Bose fellowship.

Dr. Subeer S. Majumdar
- Awarded Dr. N. N. Dastur Oration award by NDRI, Karnal, 2017.
- Awarded Manipal Association of Clinical Embryologists (MACE) award 2016 for outstanding contribution to embryology.

Dr. Pushkar Sharma
- Received J C Bose fellowship.

Dr. Rahul Pal
- Elected fellow of NASI, Allahabad.

Dr. Sagar Sengupta
- Elected fellow of the INSA.
- Elected fellow of the Indian Academy of Science (IASc), Banglore, India.

Dr. Vinay K. Nandicoori
- Awarded P. S. Sarma Memorial Award (2016) by Society of Biological Chemists.

Dr. Arnab Mukhopadhyay
- Elected as a member of the Guha Research Conference in 2017.

Dr. Sanjeev Das
- Awarded Prof. Umakant Sinha Memorial Award, Indian Science Congress Association, India, 2017.
- Awarded Prof. B. K. Bachhawat Memorial Young Scientist Lecture Award, National Academy of Sciences, India, 2016.
- Awarded National BioScience Award for Career Development, Department of Biotechnology, Govt. of India, 2015 (announced in 2016).

Dr. G. Aneeshkumar Arimbasseri
- Received Ramanujan fellowship in 2016-17.

Dr. Nimesh Gupta

- Awarded The Martin Villar Award for pre-clinical research in Thrombosis and Haemostasis by GRIFOLS SA, under the aegis of world Federation of Hemophilia.
- Awarded Global Health Travel Award of Bill and Melinda Gates Foundation.
- Received Ramalingaswami Fellowship from Department of Biotechnology, Govt. of India.

Ph.D DEGREES AWARDED TO NII SCHOLARS

Twenty-six 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:

S. No.	Students Name	Topic of Research	Guide
1	**Banoth Balaji**	Exploring the of non- canonical NF-κB signaling in modulating the canonical NF-κB responses	Dr. Soumen Basak
2	**Satya Pal Arya**	Role of micro RNA in spermatogenesis	Dr. Subeer S. Majumdar
3	**Abhisek Bhardwaj**	Understanding regulation and function of SIRT6	Dr. Sanjeev Das
4	**Anupama Singh**	A study of transcriptional modulators downstream of the nutrient sensing pathways in *Caenorhabditis elegans*	Dr. Arnab Mukhopadhyay
5	**Manpreet Kaur**	Understanding the role of replication proteins in mitosis	Dr. Sandeep Saxena
6	**Kapil Mangalani**	Bone remodelling during hyperhomocysteinemia & development of novel therapetutic modality	Dr. Sarika Gupta
7	**Jitender Kumar Verma**	Manipulation of host rab GTpase(s) by *Leishmania donovani* in macrophages	Dr. Amitabha Mukhopadhayay
8	**Richa Jalodia**	Identification and characterization of nivel host factors interacting with Japanese encephalitis virus non-strucura protein NS2A	Dr. Akhil C. Banerjea Co-Guide Dr. Sudhanshu Vrati
9	**Farhat Parween**	A study on the role of membrane prohibitin in cell signaling	Dr. Ayub Qadri
10	**Rohit Verma**	Understanding the role of signal transduction pathways in dendritic cell development	Dr. Prafullakumar B. Tailor
11	**Ruchika**	Unraveling the role of CbpL, a putative surface protein of *Streptococcus pneumonia* in host- pathogen interaction	Dr. Devinder Sehgal
12	**Surabhi Jaiswal**	Dissection of molecular mechnanisms involved in neuronal differentiation and death	Dr. Pushkar Sharma
13	**Varkhande Suraj Risha**	Investigation of the molecular perturbations involved in vitiligo aetiopathogenesis	Dr. Sangeeta Bhaskar Co-Guide Dr. Rajni Rani
14	**Namita Gupta**	Studies on role of heat shock protein 70-2 in ovarian carcinogenesis	Dr. Anil K. Suri

S. No.	Students Name	Topic of Research	Guide
15	**Smriti Parashar**	Identification and functional characterization of COPII machinery *Leishmania*	Dr. Amithabha Mukhopadhyay
16	**Sudha Saryu Malhotra**	Molecular characterization of the underlying mechanisms associated with trophoblastic BeWo cell fusion	Dr. Satish K.Gupta
17	**Arundhoti Das**	Analysis of the factors regulating T-helper cell differentiation and survival	Dr. Vineeta Bal
18	**D'souza Lucas Lionel**	Analysis of unswitched and isotype switched murine B cell memory subsets	Dr. Anna George
19	**Jairam Meena**	Formulation and immunological evaluation of polymer paticle entrapped flagellin conjugated Vi polysaccharide antigen	Dr. Amulya K. Panda
20	**Mansi Shukla**	A transgenic approach to determine role of certain genes expressed by Sertoli cells in regulation of germ cell development	Dr. Subeer S. Majumdar
21	**Rameez Raja**	Role of cell survival pathways in HIV-1 pathogenesis	Dr. Akhil C. Banerjea Co- Guide Dr. Prafullakumar B. Tailor
22	**Raina Priyadarshini**	Investigating the role of BLM helicase in regulating the $SCFF^{bw7}$ E3 ligase dependent proteasomal degradation of the AP-1 transcription factor family members	Dr. Sagar Sengupta
23	**Renu Balyan**	Analyzing determinants of variability in T cell responses	Dr. Satyajit Rath
24	**Rohit Singh Dangi**	Structural and functional studies on an AcyI-CoA Binding Protein (LmjF.17.0620) of *Leishmania major*	Dr. Monica Sundd
25	**Swarnendra Singh**	Stuides on the role of heat shock protein 70-2 in renal cell carcinogenesis	Dr. Anil K. Suri
26	**Alla Singh**	Investigation of the catalytic mechanism of Ribonuclease P of *Mycobacterium tuberculosis*	Dr. Janendra K. Batra

LECTURES AND SEMINARS

FOUNDATION DAY LECTURE

On 6th October 2016, the 30th Foundation Day of NII was celebrated at the Institute. **Prof. K. R. Shivanna**, Formerly Professor and Head, Department of Botany, University of Delhi & INSA Honorary Scientist, Ashoka Trust for Research in Ecology & the Environment, Bengaluru was invited as a Guest of Honour. He delivered a lecture on "**Galapagos Islands and Darwin's Theory of Evolution**".

DR. S. RAMACHANDRAN MEMORIAL LECTURE 2016

Dr. S. Ramachandran Memorial Lecture was organized at the Institute on 9th September 2016. The lecture was delivered by Dr. Manju Sharma, Former Secretary, Department of Biotechnology.

The lecture was attended by DBT Secretary, Professor K. VijayRaghavan, Mrs. Ramachandran, scientists, eminent researchers and DBT officials. Dr. T. Ramasami, former DST Secretary was the guest of honour for the programme.

As the first Secretary of the Department of Biotechnology, Dr. Ramachandran was responsible for creating a vibrant research ecosystem across the country focusing on all the important components of human resource and infrastructure and research capacities. India was one of the first countries to boast of a separate Department of Biotechnology in the Government.

Dr. Manju Sharma and Prof. K.VijayRaghvan

LECTURES

Prof. K. Muniyappa (Department of Biochemistry, Indian Institute of Science, Bengaluru) delivered a lecture on "**Molecular Insights into Meiotic Chromosome Pairing from Single Molecule Analysis**" on 27th March, 2017.

Prof. K. Muniyappa delivering a lecture on "Molecular Insights into Meiotic Chromosome Pairing from Single Molecule Analysis".

A lecture on "**HIV-1 and Influenza Immunogen Design**" by **Prof. Raghavan Varadarajan** (Chairman, Molecular Biophysics Unit, Indian Institute of Science, Bangalore) was organized on 21st March, 2017. After the lecture, Prof. Varadarajan interacted with the faculty members and research scholars.

NII Director, Dr. Anil K. Suri honoring Prof. Varadarajan after his lecture on "HIV-1 and Influenza Immunogen Design".

SEMINARS

S. No.	Topic	Presented by	Date
1	Immunosenescence: a cause or consequence of aging?	**Dr. Anis Larbi** SlgN, ASTAR, Singapore	4th April 2016
2	Recapitulating adult human immune traits in laboratory mice by normalizing environment	**Dr. Lalit Beura** University of Minnesota, Twin Cities,USA	6th April 2016
3	Skin homeostasis: Balancing act of cell death and inflammation	**Dr. Snehlata Kumari** Institute of Genetics, CECAD Research Centre, University of Cologne, Germany	22nd April 2016
4	Regulation of cell cycle and replication in mammalian cells	**Dr. Samarendra K Singh** University of Virginia Charlottesville, Virginia, USA	16th May 2016
5	Pathophysiological insights into human complex genetic diseases and traits from large scale genetic association studies	**Dr. Bratati Kahali** Department of Computational Medicine and Bioinformatics University of Michigan Ann Arbor, Michigan, USA	18th May 2016
6	Gene- drive technology and its applications	**Prof. Ethan Bier** Cell and Development Biology, University of California, USA	23rd May 2016
7	Thymidylate Synthase: From biophysical to structural and mechanistic studies	**Prof. Amnon Kohen** Professor of Chemistry and Molecular and Cellular Biology, The University of lowa, lowa City, USA	1st June 2016
8	Unraveling the stereochemistry and dynamics of the substrate binding site of bacterial peptidyl-tRNA hydrolase	**Dr. Ashish Arora** Central Drug Research Institute (CDRI), Lucknow	9th June 2016
9	Linc'ing' RNA to DNA repair	**Dr. Vivek Sharma** Khorana Nirenberg Fellow, NCI, NIH, USA	13th June 2016
10	Alpha-SNAP dependent cation flux and ATP signaling regulate CD4 T Cell activation and regulatory T cell development	**Dr. Monika Vig** Washington University School of Medicine, St. Louis, USA	28th June 2016
11	Regulation of inflammatory responses – New Players and pathways	**Dr. Chandrashekhar Pasare** UT Southwestern, Dallas, USA	07th July 2016

S. No.	Topic	Presented by	Date
12	Latest trends and development in segment of high content screening	**Mr. Stefan Letzsch,** Application Engineer HCS, Perkin Elmer, USA	8th August 2016
13	Mosquito immunity to human pathogens: opportunities for disease control	**Dr. George Dimopoulos** Johns Hopkins Bloomberg, School of Public Health, USA	10th August 2016
14	Different note: NF-kappaB activation signaling: a tale of two kinases	**Prof. Gourisankar Ghosh** Department of Chemistry and Biochemistry, University of California, San Diego, USA	22nd August 2016
15	Role of the endoplasmic reticulum in de novo peroxisome biogenesis	**Prof. Suresh Subramani** Division Biological Sciences, University of California San Diego, USA	29th August 2016
16	Role of regulatory T cells in immune tolerance of pregnancy	**Prof. Sarah. A Robertson** Director, Robinson Research Institute, School of Medicine, University of Adelaide, Australia	22nd September 2016
17	Tales of two novel classes of nucleic acids: Microdnas and trfs	**Dr. Anindya Dutta** Harry F. Byrd Professor and Dept. of Biochemistry and Molecular Genetics, Professor of Pathology, University of Virginia School of Medicine Charlottesville, VA, USA	6th October 2016
18	From fruit fly genetics to human cancer	**Prof. L.S. Shashidhara** Dean (Research & Development) and Chair of Biology Indian Institute of Science Education and Research (IISER), Pune	7th October 2016
19	What's right with phagosomal pathogens?	**Dr. Subash Sad** Director of the Microbiology Graduate, Program and Professor, Department of Biochemistry, Microbiology and Immunology Faculty of Medicine, University of Ottawa, Canada	24th October 2016
20	G-quadruplex ligands are more than simple telomere targeting agents	**Dr. Jean-Francois Riou** Institute de Biologie, Paris, France	25th October 2016

S. No.	Topic	Presented by	Date
21	Positioning the plane of cell division: the role of cortical centralspindlin	**Dr. Angika Basant** University of Chicago, Chicago, USA	3rd November 2016
22	Bactericidal permeability increasing protein and its function in bacterial pathogenesis	**Prof. Dipshikha Chakravortty** Humboldt Fellow, Department of Microbiology and Cell Biology, Associate Faculty, Biosystems Science and Engineering, Indian Institute of Science, Bangalore	9th November 2016
23	Genetic instability and proliferation and survival pathways in RB-related malignancies	**Dr. Suresh Jhanwar** Memorial Sloan Kettering Cancer Center, New York, USA	23rd November 2016
24	Immune regulation of autoimmune demyelinating disease	**Prof. Nitin Karandikar,** MD, Ph.D University of Iowa Healthy Care/ Carver, College of Medicine, Iowa, USA	30th November 2016
25	Industrialization of biology: making nature accessible and affordable	**Dr. Ajikumar Parayil** MANUS BIO, 1030 Massachusetts Avenue, Cambridge, MA 02138, USA	13th December 2016
26	Remodeling immune responses to improve therapeutic efficacy in murine models of cancer and infectious disease	**Dr. Purnima Dubey** Director, Cell and Viral Vector Laboratory Wake Forest School of Medicine, NC, USA	14th December 2016
27	Constructing and maintaining an apical domain in polarized cells	**Dr. Raghu Padnijat** National Centre for Biological Sciences, Bengaluru	16th December 2016
28	Genomics-driven immunotherapy of human cancers: A very personalized medicine	**Prof. Pramod Srivastava** University of Connecticut, Farmington, USA	27th December 2016
29	Calcineurin, an unexpected integral component of the T cell receptor signaling machinery	**Dr. Debjani Dutta** Laboratory of Immune Cell Biology National Cancer Institute, National Institutes of Health (NIH) Bethesda, Maryland, USA	10th January 2017

S. No.	Topic	Presented by	Date
30	Novel role for a nucleoporin in microRNA pathway	**Dr. Jomon Joseph** Scientist National Centre for Cell Science, Pune	11th January 2017
31	Studies on the effects of Hepatitis C Virus infection and Alcohol use on immune dysfunction and identification of biomarkers of liver damage	**Dr. Banishree Saha** Department of Polymer Science and Engineering, University of Massachusetts, Amherst, MA, USA	20th January 2017
32	Telomerase reactivation in cancer: mechanistic insights and therapeutic opportunities	**Prof. Vinay Tergaonkar** Principal Investigator Institute of Molecular and Cellular Biology, Singapore	27th January 2017
33	Harnessing the power of precision medicine to enhance responsiveness to cancer immunotherapy	**Dr. Akash Patnaik** M.D.,Ph.D, M.M.Sc. Director, Laboratory for Developmental Therapeutics University of Chicago Comprehensive Cancer Centre, Chicago, IL, USA	22nd February 2017
34	Critical steps in red blood cell invasion of the malaria parasite	**Prof. Tim Gilberger** Bernhardt Notcht Institute, Hamburg, Germany	24th March 2017

CONFERENCES/SYMPOSIA/ WORKSHOPS

Poster Competition on National Science Day

National Science Day (February 28th, 2017) was celebrated with great fervor on campus. The day constitutes a major science festival in NII, during which Ph.D students present posters describing their research.

Ph.D Scholars presenting their posters on National Science Day.

Indo-French Symposium on "Pathogens and Host Response"

An Indo-French symposium on "Pathogens and Host Response" was held from 10th to 12th August, 2016. The event was held under the auspices of the Indo-French International Associated Laboratory in the areas of Systems Immunology and Genetics of Infectious Diseases (LIA SIGID), which was created in November 2012 by the CNRS and DBT to integrate multi-disciplinary teams dedicated to the study of immunology and genetics of infectious diseases. Dr. Pierre-André Cazenave (The University of Pierre and Marie Curie, France) presented the Inaugural Lecture and Prof. George Dimopoulos (Johns Hopkins School of Public Health, USA) delivered the Plenary Talk. Scientists and students from different labs in India and France participated.

Dr. Pierre-André Cazenave delivered the Inaugural Lecture on the occasion of the Indo-French symposium on "Pathogens and Host Response".

"Visualizing Science" Workshop

A stimulating two-day workshop was conducted on 30th-31st March, 2017 on visual communication tools for scientists. Inaugural remarks were presented by Prof. K. VijayRaghavan (Secretary, Department of Biotechnology, Government of India) and Dr. Anil K. Suri. Hands-on training with a photographer, an illustrator, a film maker and a virtual reality expert formed part of the workshop

Prof. K. VijayRaghavan (Secretary, Department of Biotechnology, Government of India) with Dr. Anil K. Suri during the Workshop "Visualizing Science".

Science Setu activities

To contribute to the national goal of encouraging more and more bright young minds to take up the pursuit of science and technology as a career option, NII formulated a detailed programme to connect itself to undergraduate colleges, working out the principles, scope and mode of implementation in informal consultation with several colleges of the University of Delhi. The programme was named Science Setu. The following are the activities carried out in the year 2016-2017 under the auspices of Science Setu program:

1. NII provided opportunities to 15 students for short-term training during the reporting period.
2. Dr. Sagar Sengupta and Dr. Devinder Sehgal gave talks at the SGTB Khalsa College, University of Delhi on 23th March, 2017. The audience comprised of undergraduate students of various colleges of University of Delhi. It was followed by half day Lab visit in the labs of Dr. Sagar Sengupta and Dr. Devinder Sehgal on 24th March, 2017.
3. NII signed a Memorandum of Understanding (MOU) with Manav Rachna International University on 26th October, 2016.
4. On 27th October, 2016, a lecture series was presented under the Science Setu program in which Dr. Chandrima Shaha delivered a lecture on "A career in science: The pleasure of solving mysteries" and Dr. Rahul Pal delivered a lecture on "The immune system in health and disease".


FR-E² SuperBright

INFRASTRUCTURE

EQUIPMENT

While most of the routine equipment is available in various laboratories of NII, some high-end instruments facilities are shared by various research groups and their collaborators. The equipment in these facilities includes: Mass Spectrometer, NMR Spectrometer, Confocal Microscopes, Atomic Force Microscope, Scanning and Transmission Electron Microscopes, High Throughput DNA , sequencer flow cytometers, Dual wavelength X-ray Generator, X-Ray Crystallography, Flow Cytometers, Whole Body Imager, CD Spectroscopy, Surface Plasmon Resonance System and Amino Acid Sequencer.

BSL-II FACILITY

There 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 supply defined strains of mice and rats to the scientific community of the institute. Apart from the breeding and maintenance of defined strains of mice and rats, undefined stocks of other laboratory animals are also bred. 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). Mice in the Foundation Stock consist of pedigreed identified pairs which are reared in restricted area under a barrier facility. The animals from this colony provide breeding pairs for the pedigreed expansion stock colony as well as replace those pairs in the Foundation Stock which have completed their breeding life. The animals in the Production Stock consist of randomly-mixed animals from Foundation as well as Expansion colonies which are raised in the conventional manner.

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, inbred strains are propagated and replaced periodically in a manner that minimizes the genetic drift and inbreeding depression. Random samples from some of the breeders of the Foundation, Expansion and Production stocks are monitored with the help of a few microsatellite and biochemical markers to ensure 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.

The health monitoring program includes the regular screening for pathogens (including hepatitis virus, parvovirus, norovirus, and streptococcus pneumoniae, mycoplasma and sendai virus) using Elisa and PCR. Bacterial pathogens such as Pseudomonas aeruginosa, Streptobacillus moniliformis, Bordetella, Bronchiseptica, Citrobacter rodentium, Pasteurella pneumotropica, Staphylococci and E. coli are screened using culture, biochemical and PCR. Faecal samples are randomly assessed for the presence of endoparasites by the sedimentation method for the presence of syphacia and aspicularis species. Also, periodic FACS analyses are carried out on immunodeficient mice for their leakiness.

The health quality procedures which are implemented to prevent the transmission of infection between cages include careful handling of animals, washing (using automated cage and bottle washers), use of sterilized corn cob bedding, autoclaving of cages, and use of acidified autoclaved drinking water. Breeding colonies are maintained in IVC systems of international standard. The recommended schedule of medication is strictly followed to prevent infections.

PRIMATE RESEARCH CENTRE

The National Institute of Immunology has a Primate Research Centre which provides services to various investigators of the Institute for basic, pre-clinical and toxicological research. Rhesus monkeys (Macaca mulatta) are bred and maintained in the Primate Research Centre.

Group mating is carried out for the production of healthy animals. This helps in providing animals of known age and parentage. Large open pens are employed to maintain semi-natural conditions, where food and water is provided ad libitum. Infants are weaned at the age of six months and 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 pubertal age. To prevent cross-cage contamination, all cages are washed routinely by scrubbing with soap and are painted once a year. De-worming of the colony is done at least once a year. To arrest outbreaks, the routine TB tests are performed. Chest x-rays are carried out to assist in diagnosis. Sick animals are isolated and treated according to international norms, after pathological investigations and veterinary consultation. To treat 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 the appropriate content of fat, carbohydrate and vitamin are provided to the monkeys ad libitum. In addition to this bread, germinated gram, vegetables and/or fruits are also given daily. For change of taste, occasional feasts like bread with sauce or jiggery-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 connected to a flexible protective hose-pipe at the top of each cage. Steel nozzles with a Teflon interior are fitted at the tip of these hosepipes for continuous access to drinking water. Regularly meetings are held with the Staff members to endure constant mentoring. Attendants are provided with overalls, jackets, pants and footwear for use during animal handling and cleaning. Use of gloves and mask is mandatory. A booster vaccination of TT is administered to Staff members once every year. Staff members also receive booster shots of anti-rabies vaccine when required. TB tests and chest x-rays of both Staff members and associated security personnel are performed at regular intervals. As a preventive measure, personnel carrying injuries of any sort are not permitted to carry out animal-associated 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 the area surrounding the building by using disinfectants and insecticides. To prevent colonization of microbes, sewer channels and room tiles are routinely cleaned.

Major surgeries are performed in a well-equipped operation theatre, whereas minor surgeries (involving cuts and wounds) are performed in an adjacent animal prep. room. Technical expertise for surgery, immunization, blood sampling biopsies, electro-ejaculation and fertility studies is extended to investigators. Surgical linen is washed in a washing machine. An autoclaving facility for surgical equipments and accessories exists within the building. A research laboratory is situated in the Centre for primate-related research.

The Centre ensures that all experimental procedures involve minimum stress to the animal. Wherever unavoidable, appropriate medication is administered to reduce pain. A constant effort is made to keep the animals in comfortable and stress-free environment as per 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.

Clearance of research proposals by CPCSEA (after primary clearance from the Institutional Animal Ethics Committee, comprising of scientists from various fields of expertise and a member of CPCSEA) is a necessary requirement for conducting research on primates.

The macaques at the Centre have contributed to research related to infectious diseases, reproduction, endocrinology, immunology and contraception.

Administrative and Supporting Staff with Dr. Amulya K. Panda, Director (Additional Charge)

Establishment, Personnel and General Administration Services

The Division continues to provide key support through relentless efforts for optimally utilizing and integrating 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 etc. Also handle staff welfare, post retirement dispensation, preparation and submission of periodic reports to the administrative ministry, and handling Parliament Questions. To bolster the capabilities and enhance productivity, the Institute conducts periodical training for its Administrative 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.

The efforts initiated in the last reporting period for digitizing employee's records for implementing e-governance was continued further. The e-governance 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 and 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 a lifeline for research activities. Special emphasis is laid on the 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 and than distribute the material 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 the 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) Supply, Installation, Testing & commissioning of Boom Barrier at NII. ii) Renovation work of Community Facility Block at NII. iii) Setting up of New Laboratories & Offices. iv) Servicing/ repairing of various DG Sets to maintain back up supply in healthy condition. v) Miscellaneous works in newly allotted lab space at NII. vi) Supply, Installation, Testing & commissioning of Split Air Conditioning Units in Laboratories at NII. vii) Installation of LED lighting fixtures at NII. viii) Miscellaneous works at NII. ix) Providing & fixing of vitrified tiles at Ist floor in the lecture hall at NII.

The department is currently working on the following projects:

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

Instrumentation Section has been taking care of - Repair / Maintenance of Laboratory Equipments, telecommunication facilities and requirements, LN_2 plant operation & maintenance and disposal of obsolete equipments / items.

Instrumentation Section has also been managing technical requirements including operation/ maintenance of Audio-Video setups in Auditorium, conference hall, seminar halls etc. during seminars, conferences.

Library and Documentation Services

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

Library has developed Institutional Repository to collect, store and preserve its institution's research outputs for long-term digital preservation purposes, which in return will strengthen integrated research and enhance research communication networks in the field of Immunology and life sciences.

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 desktops of the scientists. The E-journals are available under NII subscriptions and DeLCON consortium project to NII members on intranet/LAN. Library has provided number of value added services to scientists. Library is 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-Ph.D registration courses, Doctoral Committee meetings, Academic Committee meetings, Fellowship of scholars etc. The Institute allowed the research fellows who has enrolled their fellowship from reputed Institutes such as Indian Institute of Science Bangalore (DBT-RA), ICMR (SRF/RA), NPDF, DST- Inspire Faculty, DST (WOS) and CSIR (SRA/RA). The Institute also impart short-term training to the fellows sponsored by the Indian 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, workshops, 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. Staff employed in sensitive areas prone to corruption have been rotated periodically. 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.

Dr. Devinder Sehgal (CVO) addressing the audience during Vigilance Awareness Week

'Vigilance Awareness Week' was observed in the Institute from October 31, 2016 to

November 05, 2016 during which a pledge to fight against corruption was taken on October 31, 2016. Shri M. C. Panda, Former Additional Secretary, Ministry of Commerce and presently a Consultant with UNICEF gave a talk on 'Public Participation in Promoting Integrity and Eradicating Corruption' as a part of the Vigilance Awareness Week 2016.

Computer Centre

Computer Centre has been providing all Information Technology related supports which involve managing switches and Wi-Fi controllers in a 700 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 centre staff facilitates day to day trouble shooting, maintenance and anti-virus support of about 800 PCs and other peripheral devices. In addition Computer centre 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.

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 a competitive basis after an examination and interviews.

The Ph.D Programme of the Institute was launched in the academic year 1986-87. Since then the Institute has admitted a total of 667 students in 31 batches. So far 389 students have been awarded the Ph.D degree including 26 who obtained the degree in the academic year 2016-17. Many others are at various stages of their research work for the degree.

In addition, the Institute accepts students from various Universities/Institutions as Summer Research Fellowship Awardees 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.

PUBLICATIONS

One hundred and twelve research papers by the scientists and scholars of the Institute were published this year in different areas of research being conducted at the Institute. Of these publications, one hundred and four were published in journals as peer-reviewed research papers and remaining eight papers were published as reviews/ proceedings. The complete details of these papers including author(s), title, journal name, volume, year, pagination or name of the conference, name of the book, their publishers etc. are available.

PATENTS AND TECHNOLOGY TRANSFER

The Institute has a policy of protecting the intellectual property rights on inventions made within its laboratories. Early research leads are evaluated for commercial viability and patentability. The Institute files applications first in India and when necessary, at patent offices in other countries. During the year under report the Institute has filled two patents and secured four granted patents. The Institute also has one Technology transferred – "Process for obtaining bioactive protein from inclusion bodies" has been transferred to EPR Centre for Cancer Research and Bioinformatics Private Limited (A Vitane Group of Company) located in Shamirpet Mandal, RR Dist-500078, Telangana.

LECTURES DELIVERED ON INVITATION/ PAPERS PRESENTED

The scientists of the Institute continued to deliver lectures including 'Keynote Addresses and Inaugural Addresses' 'Serial Lectures' etc at various institutions, conferences, symposia, workshops and training programmes in India and abroad.

LECTURES/SEMINARS BY VISITING SCIENTISTS/ GUEST INVESTIGATORS

The Institute continued to receive visiting scientists and guest investigators from all over the world. Thirty eight seminars were

organized by the Institute on different topics related to the areas of research being carried out at the Institute. Out of these, investigators from India delivered eight seminars and the scientists from abroad presented the rest. These seminars were attended not only by the scholars and scientist of the Institute but also by the investigators from other institutions. Fruitful discussions usually followed these seminars.

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 September, 2016. 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 have participated. Hindi Diwas (Hindi Day) was celebrated on 14th September, 2016 at the culmination of Hindi Pakhwara.

Dr. Akhil C. Banerjea distributing prizes on "Hindi Diwas"

Hindi Workshop on "writing notes & drafts in Hindi on the computer" was organized for the staff members to remove the hesitation for carrying out their official work in Hindi on computer.

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 articlets, research papers in Hindi on Scientific and Technical subjects was also implemented in the Institute.

FOUNDATION DAY LECTURE

On 6th October 2016, the 30th Foundation Day of NII was celebrated at the Institute. Prof. K.R. Shivanna Formerly Professor and Head, Department of Botany, University of Delhi & INSA Honorary Scientist, Ashoka Trust for Research in Ecology & the Environment, Bengaluru was invited as a Guest of Honour. He delivered a lecture on "Galapagos Islands and Darwin's Theory of Evolution".

ANTI-TERRORISM DAY, SADHBHAVNA DIWAS AND MARTYRS' DAY

Anti-Terrorism Day was observed by all employees of the Institute on 20th May 2016 (Friday) (21st May 2016 being weekly off (Saturday) 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 19th August 2016 (Friday) (20th August 2016 being weekly off (Saturday) 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'.

Martyrs' Day was observed on 30th January, 2016 in the memory of those who gave their lives in the struggle for India's freedom. Two Minutes silence was observed at 11.00 AM.

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, 1 vacancy reserved for Scheduled Castes and 4 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 current reporting period, one Group 'C' vacancy reserved for PWD-Hearing Impairment was filled by Junior Assistant-I, who has since joined the Institute.

INDEPENDENCE DAY CELEBRATION

Independence Day was celebrated in the Institute on 15th August 2016. 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.

FAREWELL TO Ph.D STUDENTS

A farewell function of the 2011 batch of Ph.D Students was held at the Institute on 24th June, 2016.

RTI ANNUAL RETURN INFORMATION SYSTEM (2016-2017)
ANNUAL RETURN FORM

Ministry /Department /Organization: Department of Bio-Technology (National Institute of Immunology), New Delhi-110067

Year 2016-2017 (upto March 2017)
Insert Mode (New Return)

		Progress in 2016-17			
	Opening Balance as on 01/04/2016	**Received during the year (including cases transferred to other Public Authority)**	**No. of cases transferred to other Public Authority**	**Decisions where request/appeals rejects/appeals rejected**	**Decision where requests/appeals accepted**
Request	200	29	0	0	29
First Appeals	1	0	0	0	0

No. of Cases where disciplinary action taken against any Officer	0

No. of CAPIOs designated	**No. of CPIO designated**	**No. of AAs designated**
	1	**1**

No. of times various provisions were invoked while rejecting request													
Relevant section of RTI Act 2005													
Section 8 (1)										Sections			
a	b	c	d	e	f	g	h	I	j	9	11	24	Others
0	0	0	0	0	0	0	0	0	0	0	0	0	0

	Amount of Charges Collected (in Rs.)	
Registration Fee Amount	**Additional Fee & Any other charges**	**Penalties Amount**
130	6	0

Last date of Uploading the Pro-active Disclosures on the website of PA	(Format 10/04/2017)

Name of the person who is entering/updating data	Dr. Kanwaljeet Kaur
Designation of the person who is entering/updating data	CPIO

NATIONAL INSTITUTE OF IMMUNOLOGY
NEW DELHI

Report on Monthly Disposal of Cases
2016-2017

Year	Month	Opening Balance	Receipt	Disposal	Closing Balance	Cumulative Disposal
2016	April	200	2	3	202	202
2016	May	202	3	2	205	204
2016	June	205	1	1	206	205
2016	July	206	3	1	209	206
2016	August	209	2	3	211	209
2016	September	211	0	2	211	211
2016	October	211	1	1	212	212
2016	November	212	3	2	215	214
2016	December	215	1	1	216	215
2017	January	216	3	1	219	216
2017	February	219	4	3	223	219
2017	March	223	6	6	229	225

ORGANIZATION

NII SOCIETY

Prof. G. Padmanaban
(President)
NII Society & INSA Senior Scientist, & Innovation Advisor, BIRAC, DBT
Department of Biochemistry
Indian Institute of Science
Bangalore

Prof. K. VijayRaghavan
Chairman, Governing Body, NII & Secretary to the Govt. of India, Ministry of Science & Technology Department of Biotechnology, Block No. 2. 7th Floor, CGO Complex, Lodhi Road, New Delhi

Dr. (Prof.) Jagdish Prasad
Director General,
Directorate General of Health Services
Ministry of Health & Family Welfare, Nirman Bhawan,
New Delhi

Smt. Gargi Kaul
Joint Secretary & Financial Adviser
Department of Biotechnology
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Dr. Soumya Swaminathan
Secretary, Dept. artment of Health
Research & Director General
Indian Council of Medical Research (ICMR)
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Post Box No 4911
AIIMS Hospital Campus,
Ansari Nagar, New Delhi

Dr. Trilochan Mohapatra
Secretary (DARE) & Director General (ICAR)
Indian Council of Agricultural Research
Krishi Bhawan, New Delhi

Prof. Randeep Guleria
Director
All India Institute of Medical Sciences
Ansari Nagar, New Delhi

Prof. Ved Prakash
Chairman
University Grants Commission
Bahadur Shah Zafar Marg
New Delhi

Prof. M. Jagadesh Kumar
Vice-Chancellor
Jawaharlal Nehru University
New Delhi

Prof. V. Nagaraja
President, JNCASR
Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore

Dr. Anuradha Lohia
Vice-Chancellor
Presidency University
86/1, College Street
Kolkata

Ms. Deepanwita Chattopadhyay
Managing Director & CEO
IKP Knowledge Park
Indra Towers, 3rd Floor,
Plot No.1, Krishna Nagar Colony, Secunderabad

Shri. Chandra Prakash Goyal
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Dr. Anil K. Suri
Director (Additional Charge)
National Institute of Immunology
Aruna Asaf Ali Marg
New Delhi

GOVERNING BODY

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(Chairman)
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Directorate General of
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Ministry of Health & Family
Welfare, Nirman Bhawan
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Prof. Randeep Guleria
Director
All India Institute of Medical
Sciences
Ansari Nagar, New Delhi

Prof. Ved Prakash
Chairman
University Grants
Commission
Bahadur Shah Zafar Marg
New Delhi

Prof. M. Jagadesh Kumar
Vice-Chancellor
Jawaharlal Nehru University
New Delhi

Prof. V. Nagaraja
President, JNCASR
Jawaharlal Nehru Centre for
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Management Assistants
Mr. A.K. Dey
Mr. Dev Datt Sharma
Mr. Jagdish Mogha
Mr. Siddharth Sharma

Junior Hindi Translator
Ms. Nisha

Junior Assistant II
Mr. Mohan Lal

Drivers
Mr. Madan Lal
Mr. Mahender Singh
Mr. Satyabir Singh
Mr. Suti Prakash

Technician II
Mr. Puran Singh

Skilled Work Assistants
Mr. Dinesh Singh
Mr. Nand Lal Malakar
Mr. Rajeev Kumar
Mr. Ajay Kumar

FINANCE & ACCOUNTS

Finance & Accounts Officer
Mr. Pradeep Chawla (On Deputation)

Finance & Accounts Officer
Mr. Padam Singh Rawat

Section Officers
Mr. Rakesh Satija
Mr. Aslam Ali

Management Assistants
Mr. Suresh C. Chandel
Mr. Pradip K. Sarkar

Technician II
Mr. Brahm Dev

STORES & PURCHASE

Section Officer
Mr. Mahender Pal Singh

Management Assistants
Mr. Dharambir
Mr. Than Singh
Mr. Om Prakash

Junior Assistant I
Mr. Alam Singh
Mr. Debarshi Deb

Junior Assitant II
Mr. Daya Chand

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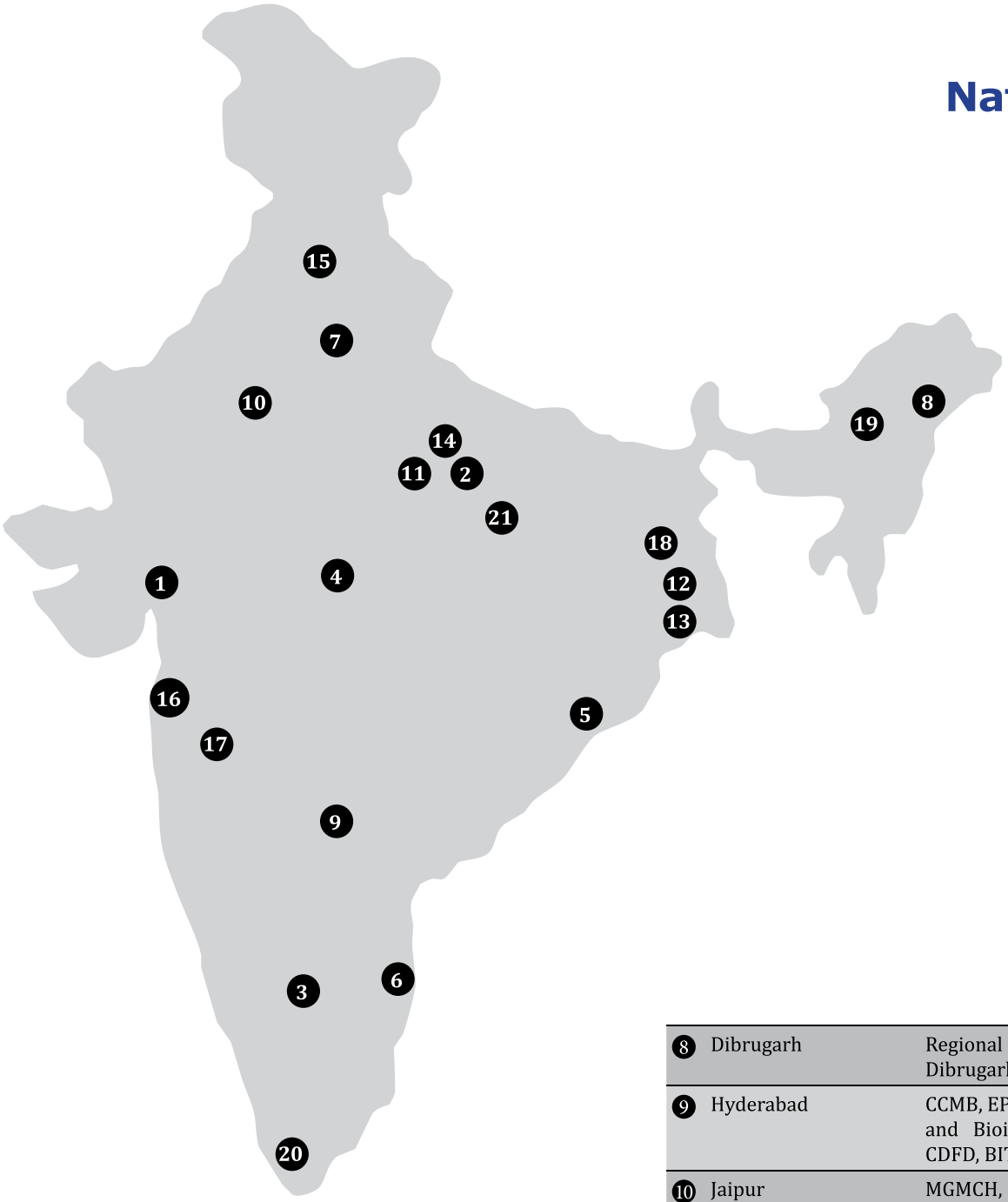
A page from history

Prof. S. Nurul Hassan, Governor, West Bengal inaugurated the Product Development Wing of the Institute (1992). Dr. S. Ramachandran, Secretary, Department of Biotechnology, Prof. G. P. Talwar, Former Director, NII and Dr. Sandip K. Basu, Director NII were present.

NII Collaborations

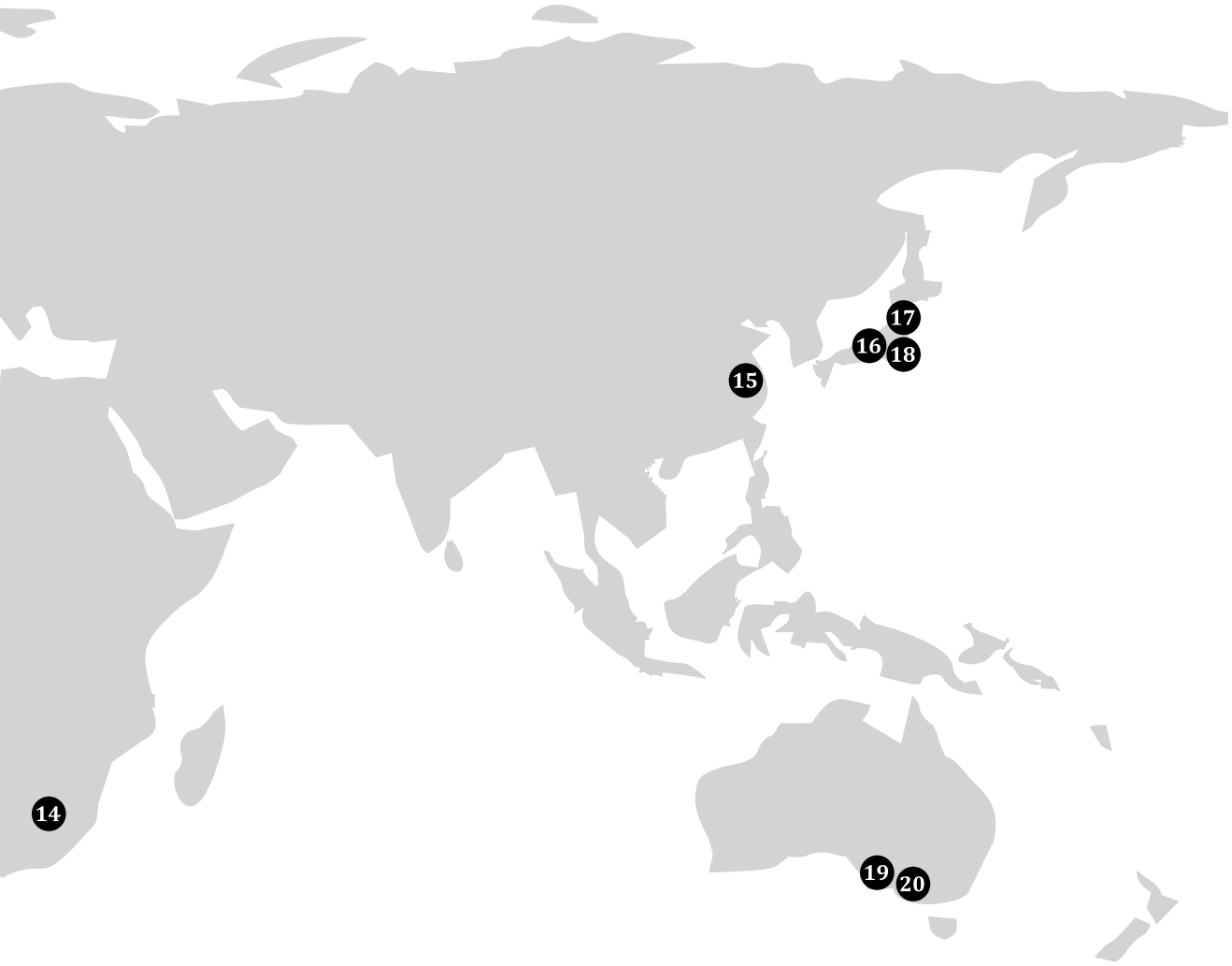
National



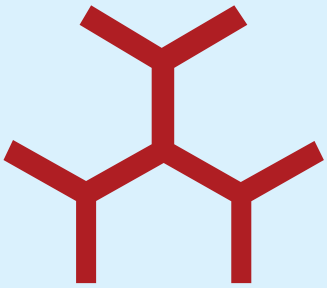
1	Ahmedabad	Nirma University
2	Allahabad	University of Allahabad
3	Bangalore	NCBS, IISc, St John's Medical College Hospital, Christian Blind Mission, IOB, St. Johns Research Institute, JNCASR
4	Bhopal	NIREH
5	Bhubaneswar	KIT, CIFA, ILS
6	Chennai	Cancer Institute Adiyar (WIA)
7	Delhi/ NCR	THSTI, AIIMS, JNU, UCMS/GTB Hospital, DU, Jamia Hamdard, NSIT, TRF, I CARE Eye Hospital, National Institute of Pathology, IITD, St. Stephen's Hospital, INMAS, NPL, ICGEB, CSIR-IGIB, IRCH, SH &VMMC, VIMHANS Hospital, RCB, Amity University,
8	Dibrugarh	Regional Medical Research Centre, Dibrugarh
9	Hyderabad	CCMB, EPR Centre for Cancer Research and Bioinformatics Private Limited, CDFD, BITS
10	Jaipur	MGMCH, Centre for Advanced Studies, University of Rajasthan,
11	Kanpur	IIT-Kanpur
12	Kalyani	NIBMG
13	Kolkata	IICB
14	Lucknow	Central Drug Research Institute, SGPGI,
15	Mohali	IISER, Mohali
16	Mumbai	TIFR, NIRRH, ACTREC,
17	Pune	CSIR-NCL, BAIF, NCCS, IISER
18	Shantiniketan	Vishwabharti University
19	Tezpur	Tezpur University, Assam
20	Thiruvananthapuram	HLL Lifecare Limited, Thiruvananthapuram
21	Varanasi	BHU Medical College

International

1	Institute for Systems Biology, Seattle Proteome Center, Seattle, USA
2	La Jolla Institute for Allergy and Immunology, La Jolla, California, USA
3	University of Arkansas, Fayetteville, USA
4	National Institutes of Health, Bethesda, USA
5	State University of New York (SUNY), Buffalo, USA
6	Columbia University, New York,USA
7	Harvard School of Public Health, Boston, USA
8	Universite de Rennes, Rennes, France



9	Imperial College London
10	Cambridge University, UK
11	Institute Pasteur, France
12	University of Geneva, Switzerland
13	Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
14	University of Pretoria, South Africa
15	Shanghai Institute of Planned Parenthood Research, P.R.China
16	Osaka University, Japan
17	Kanazawa University, Japan
18	RIKEN Brain Science Institute (BSI), Japan
19	Walter and Eliza Hall Institute (WEHI), Australia
20	Monash University, Melbourne



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