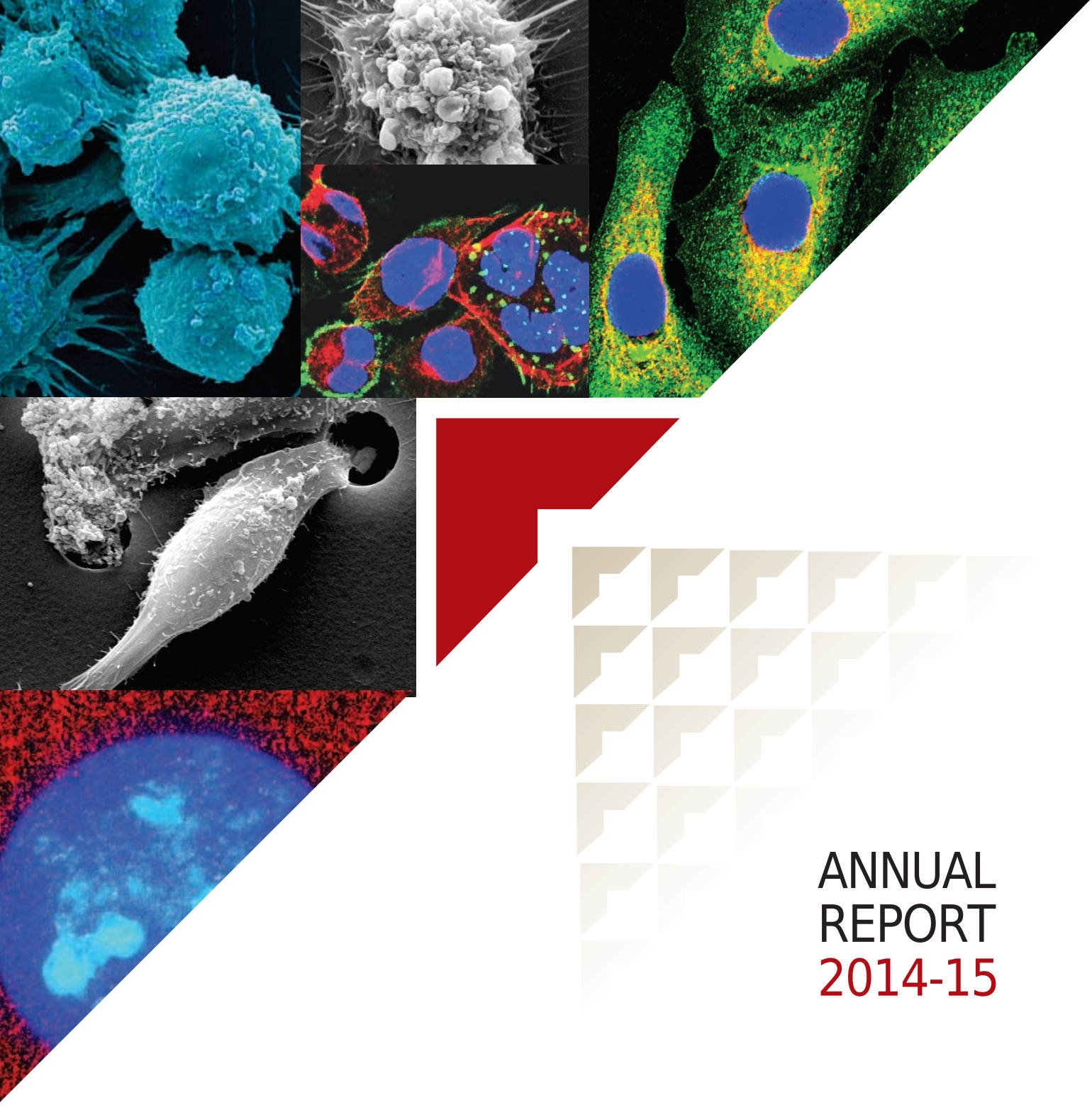
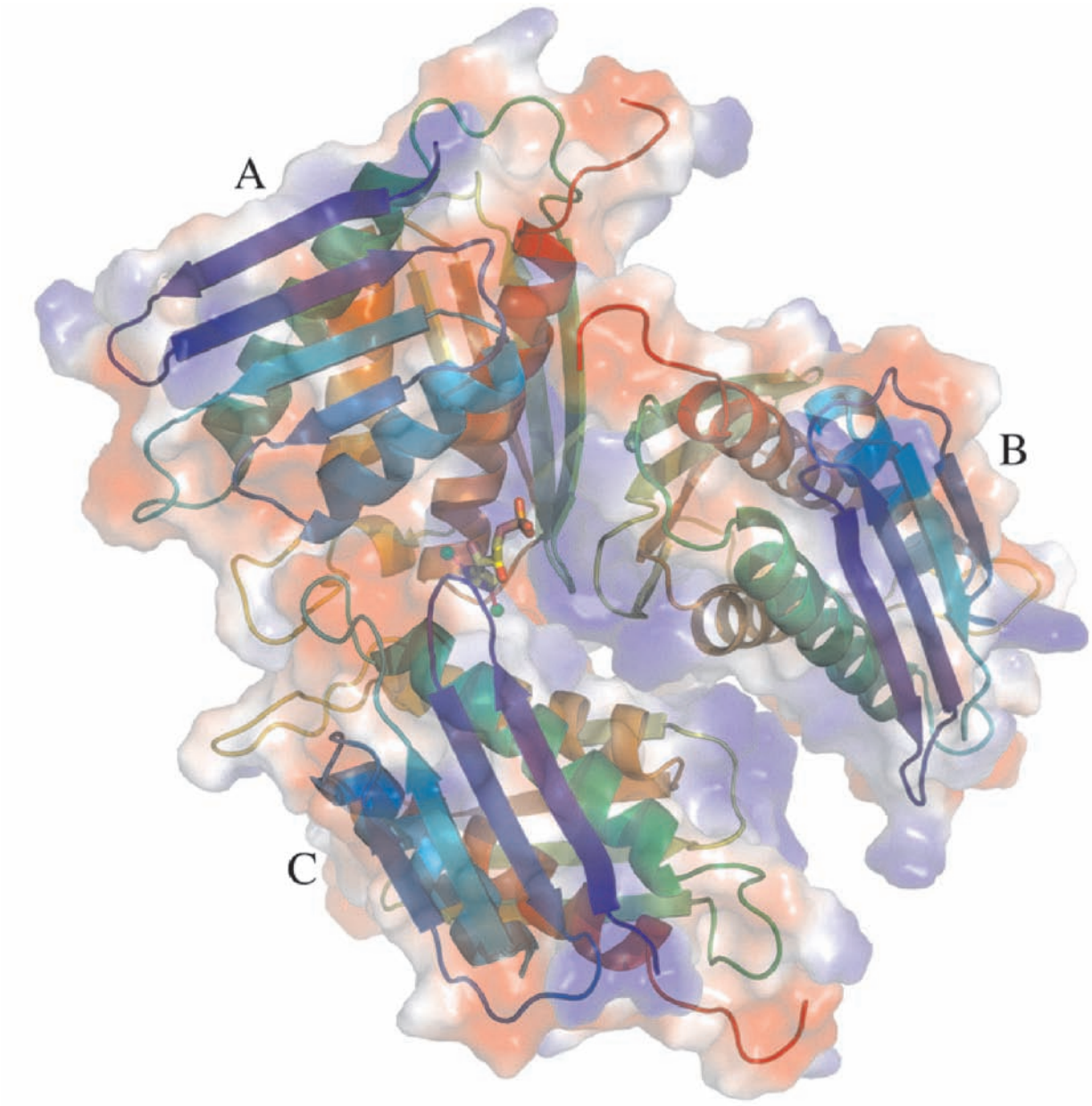


# ANNUAL REPORT 2014-15

National Institute of Immunology








One of the 24 catalytic centers of HisB formed in the interface of three subunits A, B and C

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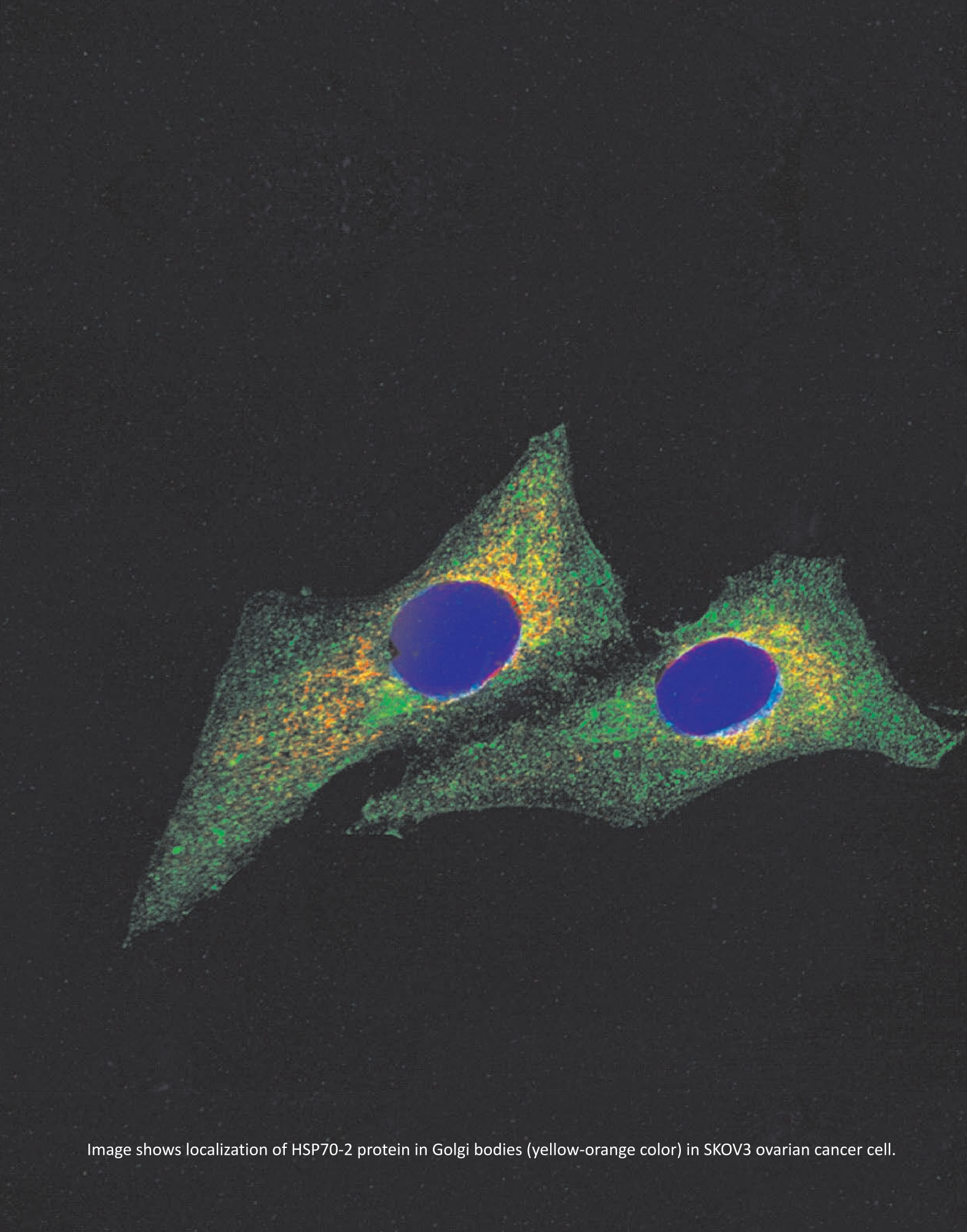
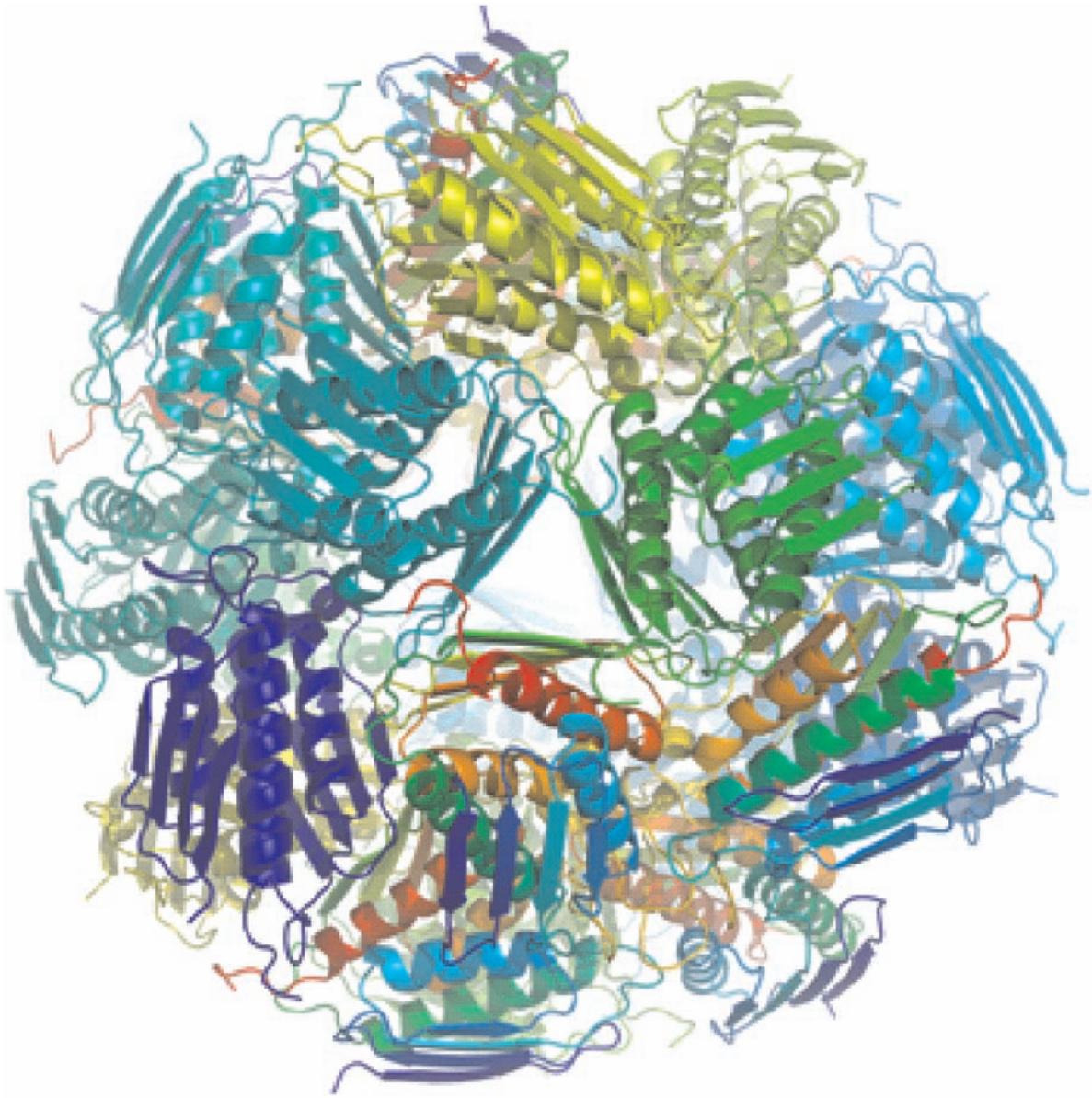


Image shows localization of HSP70-2 protein in Golgi bodies (yellow-orange color) in SKOV3 ovarian cancer cell.

# 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.



Cartoon representation of the 3D structure of the biological unit, a 24-mer assembly, of *M. tuberculosis* HisB (an imidazole-glycerol-phosphate dehydratase).



# FOREWORD

With great pride and pleasure I present the Annual Report of 2014-2015.

During the above reporting period, the institute witnessed a sizable number of significant achievements in the field of science as well as society activities. First, I would like to introduce the science and society activities that we have undertaken. Advance of science education is essential for the progress of the country and therefore it is essential to encourage young students to take up research in life sciences as a career option. To facilitate this process NII started a programme called the 'Science Setu'. NII reached out to the colleges of Delhi and ten colleges namely, Daulat Ram, Dyal Singh, Gargi, Hansraj, Hindu, Miranda House, Ramjas, SGTB Khalsa, Sri Venkateswara, Zakir Hussain, Delhi College and Institute of Home Economics signed MOU with NII to implement a programme. This programme enables NII scientists to teach in the Delhi colleges and the students to visit laboratories and do short term trainings. Science Setu was launched at a formal function at NII on March 26 of 2015. This occasion was graced as chief guest by Professor K. VijayRaghvan, FRS and Secretary, DBT who spoke on "Why we should stop studying (and have fun learning)". He emphasized that the only path to success was through hard work and love for whatever one does. Professor Raghavendra Gadagkar of the Indian Institute of Science at Bengaluru and President of the Indian National Science Academy was the Guest of Honour and delivered a talk on "Science Education Indisciplined". The programme has generated a great deal of goodwill and excitement among the academic community and enthusiastic responses have been received from colleges. As the programme extends into the future, I am sure many students will benefit from these interactions.

The institute continues to nurture its doctoral, post-doctoral and training programmes in cutting-edge biological sciences. The total number of Ph. D students was 187 in number and 25 Ph.D degrees were awarded during the reporting period. As like other years, summer trainees formed a sizable group of students who were trained in a variety of ways to do experimental science along with other long term associates and fellows. Foreign fellows also spent different periods of time in the institute for training. Efforts have taken place to provide training facilities in advanced biological sciences to developing country scientists to promote research linked training and create a network for building research collaborations. International visiting scientists and trainees

from Nigeria (NAM S&T Centre), Egypt (CV Raman Fellowship, DST) and Tunisia (CV Raman Fellowship) have availed this facility as well as a trainee from Cameroon (OWSD fellowship).

The institute continued to present regular seminars of faculty from various scientific organizations and three major workshops took place. A colloquium lecture was delivered by Dr. Michael L. Klein, Dean, College of Science and Technology, Temple University, Philadelphia, on "The Spark of Life : Understanding Nature's Ion Channel Design via Computation". The Ramalingaswami Memorial Lecture was delivered by Prof. Alexander Rudensky, Chairman, Immunology Program and Director, Ludwig Center for Cancer Immunotherapy, Memorial Sloan-Kettering Cancer Center, New York who talked on "Regulatory T CELLS and control of inflammation". On the Foundation Day, Prof. Ajay Sood from the Indian Institute of Science, Bangalore gave a lecture on "Driven and active soft matters: Fun Science". As like other years, National Science Day was celebrated on 28th February, 2015 at the Institute where Prof. Vidyanand Nanjundiah of Centre for Human Genetics Bangalore spoke on "Information transfer and the autonomy of biology". In addition to these lectures, Prof. Christian Bréchot, President, Institut Pasteur, Paris, delivered a Lecture on "Institut Pasteur: Novel Scientific and Strategic Challenges" and Prof. Philippe J. Sansonetti, head of the Unité de Pathogénie Microbienne Moléculaire at the Pasteur Institute of Paris and chair of Microbiology and Infectious Diseases at the Collège de France, delivered a Special Lecture on "Pathogenesis of *Shigella* infection, the Yin and Yang of innate immunity".

Several scientists received recognition from the academic community in the form of awards and election to scientific academies. Dr. Rajni Rani was awarded the Senior Scientist Oration Award from Indian Immunology Society and Dr. Satish K. Gupta received Prof. L. S. Ramaswami Memorial Oration Award of 2015, from Indian Society for Study Reproduction and Fertility, India. Dr. Sanjeev Das received the NASI-SCOPUS Young Scientist Award of 2014 in Biological Sciences. Dr. Sarika Gupta received Smt. Kusum Sharma Award from the Indian Academy of Biomedical Sciences. Dr. Anil Suri was awarded the Dr. T. C. Anand Kumar Memorial Oration Award from ISSRF (Indian Society for Study of Reproduction and Fertility) and citation award of Professor Rabindra Nath Chakravarti award conferred by Post Graduate Institute of Medical Education & Research. Dr. Apurba K Sau was awarded National Bio-science Award for Career Development. Director was awarded the Om Prakash Bhasin Award for Biotechnology from the Bhasin Foundation and 14th Pushpa Sriramachari Foundation Day Oration Award of the ICMR. Director was also Elected Fellow of The World Academy of Sciences (TWAS). The NII young scientists receiving recognition from the scientific community included Dr. Nirmala Jagadish who was awarded the Prof. G. P. Talwar Gold medal for Middle Career Scientist and Dr. Deepak Parashar was awarded the Prof. N. R. Moudgal Young Scientist Award conferred by the ISSRF.

NII signed a number of memorandum of understandings (MoU), one was a co-operation framework agreement signed between Universidade De Vigo, Spain and the institute. MoU was also signed between Biotech Consortium India Ltd. and the Institute. 'Research and Development Agreement' was signed with HLL Lifecare Limited for collaborative research on herbal based

microbicide. In the translational front, three national patents were filed and nine international patents were granted. A MOU was signed with Cancer Institute (WIA) Adiyar, Chennai to undertake human clinical trials for cervical cancer employing Dendritic Cell based cancer vaccine using recombinant SPAG9 protein discovered at NII. DCGI clearance has been obtained during the reporting period for conducting the clinical trials.

Important workshops were held during the last reporting period, one workshop on 'Infection and Immunoregulation' was conducted to provide a platform for interaction amongst scientists of the Institute and Singapore Immunology Network (SIgN), to identify potential areas for collaboration and another 'DBT-NCI Provocative Questions Workshop on Cancer Biology' was jointly organized by the National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services, Department of Biotechnology, Government of India for providing a platform to identify non-canonical questions in cancer research. A partnership workshop for exploration of collaborative activities was organized in collaboration with the Immunology Department, University of Toronto, Canada and International Congress on 'Embryo Implantation and Pregnancy: Intricacies and Strategies for its success' was held during the reporting period.

The institutes' efforts to do high quality basic research in disease biology resulted in a significant number of publications during the reporting period, a few of which is summarized below.

In an effort towards the development of a protein-based vaccine that is serotype independent and effective in all age groups, characterization of mouse generated IgG anti-PspA monoclonal antibodies (anti-PspA(hkR36A) MAbs) with heat-killed strain R36A (clade 2) was reported. The anti-PspA(hkR36A) MAb-dependent deposition of complement on pneumococci showed a positive correlation with passive protection against the strain WU2. All protective monoclonal antibodies showed bactericidal activity, however, not all monoclonal antibodies exhibiting bactericidal activity conferred protection *in vivo*. The protective monoclonal antibodies have the potential to be used for identification of conserved protection eliciting B cell epitopes for engineering a superior pneumococcal surface antigen A -based vaccine.

An investigation on phenotype and functionality of naïve CD4 T cells revealed that there were subsets of naïve CD4 cells that varied in the time consumed in the periphery receiving MHCII-mediated signals. As a result, alteration of phenotype and functionality via reactive oxygen species and dual-specific phosphatase activity occurred. These findings suggested the feasibility of possible pharmacological interventions for better CD4 T cell responses during vaccination of older people via either anti-oxidants or dual-specific small molecule phosphatase inhibitors.

Malaria remains a vital problem for India. Looking into the disease causing agent, the *Plasmodium falciparum*, scientists at the institute show that an atypical calcium-dependent protein kinase, has a pleckstrin homology domain adjacent to the kinase domain and two calcium-binding EF-hands at its N terminus. This enzyme interacts with PI(4,5)P2 via its pleckstrin homology domain

which may guide its subcellular localization. Disruption of this enzyme causes a marked reduction in the growth of the blood stage parasites and attenuates parasite proliferation. The protein kinase family that regulate diverse parasitic processes are considered as potential drug targets and in this context, this study is important.

Significant breakthroughs were attained with tuberculosis research that is not only a problem for the country but for the world as well. The protein kinase B of the *Mycobacterium tuberculosis* is an important enzyme comprising of an intracellular kinase domain, connected through a transmembrane domain to an extracellular region that contains four PASTA domains. Studies reported show that protein kinase B plays a critical role in mediating pathogen survival not only *in vitro* but is also essential for the survival of the pathogen in the host. The same group also showed that mycobacterial protein kinase A regulated growth defects. They also showed the importance of this enzyme for the survival of the pathogen *in vivo.* The data obtained indicate that protein kinase A and protein kinase B appear to be regulating cellular processes through divergent signaling pathways. Using molecular dynamic simulations, another group showed that mycobacterial Ser/Thr protein kinase B that needs phosphorylation for its activity, in comparison to the non-phosphorylated form, shows a differential network of crucial electrostatic and hydrophobic residues that helps the stabilization of the active conformation of the phosphorylated form.

As like the malaria and tuberculosis, AIDS remain a huge problem for India. NII's efforts in this direction of understanding the biology of the host after infection show major perturbations in ubiquitinated pools of host proteins in human T-cells. Infection based studies of T-cells show the necessity of viral protein R for reducing whole-cell ubiquitination. This is interesting because this event initiated by the virus diverts the ubiquitination machinery of the host specifically towards the degradation of specific host-restriction factors.

T cells are critical to immunity against pathogenic *Salmonella Typhi* which causes systemic infection, typhoid, in humans. Scientists at NII showed that the virulence polysaccharide Vi, which distinguishes *S. Typhi* from localized gastroenteritis-producing nontyphoidal *Salmonella* serovars, is a potent inhibitor of T-cell activation. The findings reveal a mechanism by which how *S. Typhi* behaves during establishment of typhoid and may be the T-cell immunity as well.

The anchoring of surface proteins to the cell wall in gram-positive bacteria is catalyzed by transpeptidase sortase. Sortase A of *Staphylococcus aureus* is a prototype enzyme and considered a bona fide drug target because several substrate proteins are virulence-related and implicated in pathogenesis. Studies carried out at NII offers insights about specificity, tolerance, and conformational sorting of the substrate by Sortase A. These investigations could help in the designing of newer substrates and inhibitors for this complex enzyme.

Research on aging processes has progressed at the institute using the model system of *Caenorhabditis elegans*. Investigations into signaling processes downstream to food/nutrient perception shows that within the organism MEKK-3 may function as an important

nutrient sensor and signalling component that controls metabolism. If MEKK3 is knocked down, nutrient crisis occurs initiating a DR-like state, even when food is plentiful. Rifampicin and its analog rifamycin SV reduces glycation of important cellular proteins *in vivo* and as a result increases lifespan of *Caenorhabditis elegans*. The data suggest that the dual ability to reduce glycation *in vivo* and activate prolongevity occurs through the involvement of the DAF-16, the FOXO homolog.

An interesting report from the reproductive sciences shows the role of FSH responsiveness in non-human primates. FSH acts on the testicular Sertoli cells bearing FSH receptor. Although an adult-like FSH milieu exist in infant boys and monkeys, spermatogenesis is initiated only at the onset of puberty. Studies by NII scientists suggest that down-regulation of Gαi-2 expression and partial expression of Gαs subunit and Ric8b may underlie limited FSH responsiveness of Sertoli cells during infancy. This information may help in the proper diagnosis and treatment of infertile individuals having abnormal G protein-coupled FSH-R. A vaccine with canine zona pellucida glycoprotein-3 was tried in the mouse model and has shown promising results.

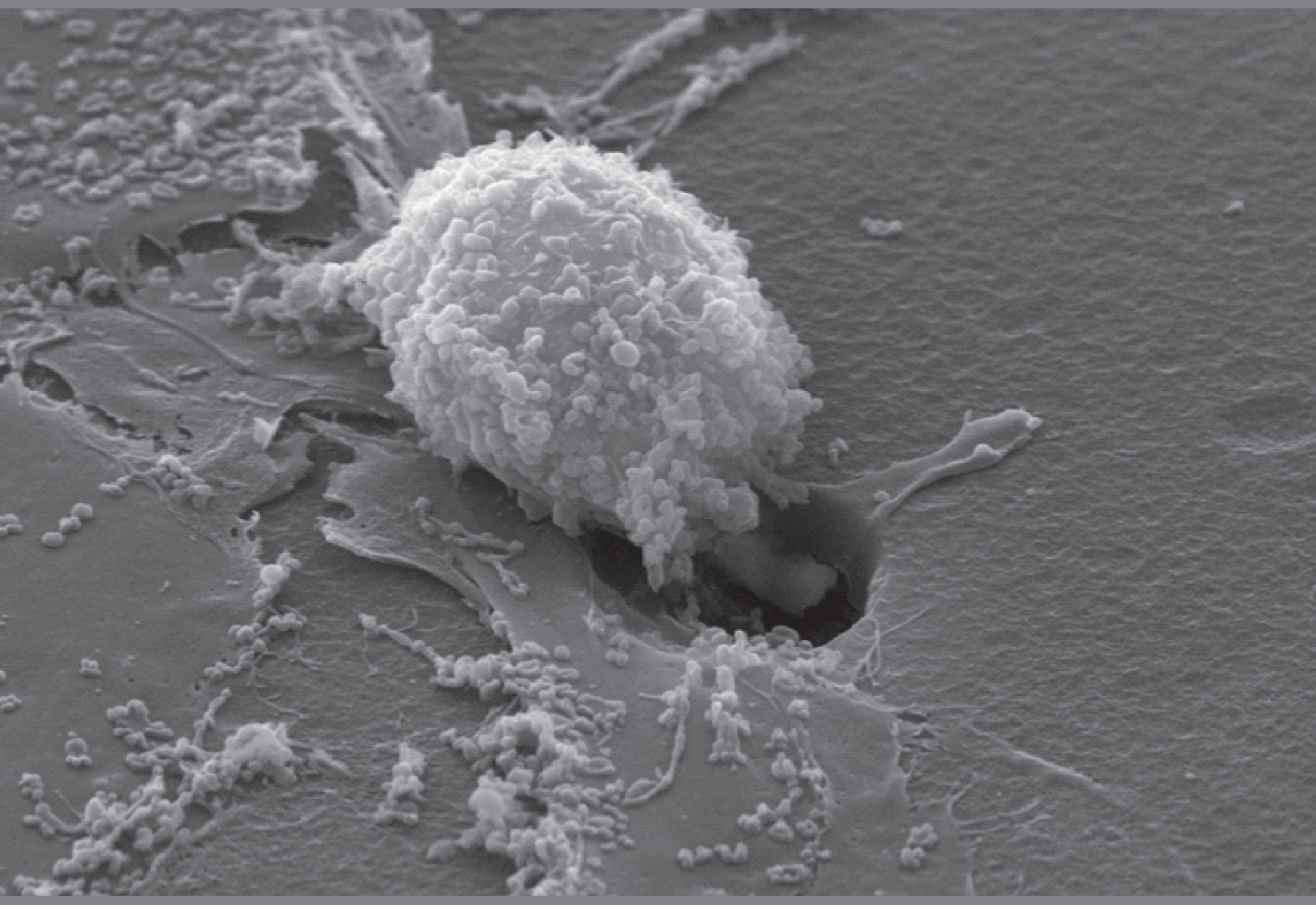
Research on understanding the basic mechanisms of cancer has thrown up interesting results on a tumor suppressor protein. The protein p73 is a member of the p53 tumor suppressor family mediating genotoxic stress response by activating cell cycle arrest and apoptosis. A pivotal role of Tyrosine99 phosphorylation of the tumor suppressor p73 was linked to its cellular levels and activity. It was shown that Tyrosine99 phosphorylation regulates p73 interaction with two proteins-TRIM28 and MED15. While TRIM28 is an E3 ubiquitin ligase essential for p73 homeostasis, MED15 acts as a co-activator integral to p73's tumor suppressing and anti-metastatic functions. Another study shows interactions of two tumor suppressor proteins Beclin-1 and p53 that determines cell fate. These interactive complexes are drug targets that can be blocked by suitable inhibitors for anti-proliferative activities.

I would like to congratulate the scientific and allied staff of the institute for a successful year. My special congratulations are due to the doctoral students, the post-doctoral research associates, research assistants, junior research fellows and senior research fellows for their enthusiastic and exciting participation in functioning of the institute. NII will strive to meet the challenges of the coming years and I am sure that with the help of all the members of the institute, growth towards a higher trajectory to meet the growing needs of human health will be possible.

All the functioning of the institute would not have been possible without the constant support from the Department of Biotechnology.

**Chandrima Shaha**
**Director**

Date: May 19, 2015



Scanning electron microscopy image shows the MDA-MB-231 breast cancer cell invading through the matrigel

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# IMMUNITY AND INFECTION

# IMMUNITY AND INFECTION


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# Satyajit Rath

## Analysis of antigen processing and presentation

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During 2014-2015, substantial progress has been made in two directions in the ongoing analyses of immune system development and function.

**A. Genesis and consequences of metabolic distinctions between closely related lymphocytic cell lineages**

In one approach, the genesis and consequences of metabolic distinctions between closely related B and T lymphocytic cell lineages are being investigated, since while they mediate distinct functions, they are very similar in lineage differentiation mechanisms. Thus, we expect similar metabolic modulations to occur during lymphocyte differentiation transitions. However, we found that B and T cell lineages show specific metabolic-functional differences. Thus, glucose uptake is higher in naive CD4 and CD8 T cells than in naive B cells, while the baseline rate of protein synthesis is higher in naive B cells than in naive T cells. These differences are functionally meaningful. Thus, naive B cells are more susceptible to cell death in response to protein synthesis inhibition, while naive T cells are more susceptible to glycolysis inhibition. We observe similar differences between immature B and T lineage stages, indicating that they are imprinted

during lineage differentiation. Thus, it appears that metabolic landscapes are differently wired even in closely analogous cell lineages. The genesis and consequences of these metabolic distinctions are of great future interest.

**B. Genetic basis of autoantibody response to anti-complement factor H (CFH) in renal disease**

A second approach addresses the hemolytic uremic syndrome (HUS), a common cause of acute kidney injury in childhood. Many if not most patients with a HUS show genetic or acquired defects in the complement system. Some 6-10% patients in European HUS cohorts show autoantibodies to an important complement regulator, complement factor H (CFH), and their occurrence is very strongly associated with mutations in genes encoding CFH-related (CFHR) proteins, specifically, CFHR1 and CFHR3. However, whether this is the case in India has not been clear.

We have therefore participated in a study on a nationwide scale anchored at AIIMS, New Delhi for the presence of anti-CFH autoantibodies in Indian children with aHUS and their relation to disease severity and course. Our study so far shows that, unlike European reports, between 50-60% of paediatric aHUS patients in India show the presence of anti-CFH auto antibodies, and ~80% of these show evidence of a homozygous deletion of the *cfhr1/3* gene/s. On the other hand, in a control set of healthy individuals, only ~10% showed evidence for a homozygous cfhr1/3 deletion, in agreement with previous reports on healthy populations. Thus, the background frequency of the cfhr1/3 deletion genotype is likely to be the same in India as elsewhere in the world; yet, its relative prominence, in association with anti-CFH autoantibodies, in paediatric aHUS is much greater in India, creating a clinical-immuno-pathological conundrum that we intend to continue to work on. Remarkably, our preliminary data also show that ~30% of anti-CFH-negative aHUS cases have a homozygous cfhr1/3 deletion, suggesting a novel auto antibody-independent pathogenetic connection between the genotype and the disease is to be examined further.

**Publications**

***Original peer-reviewed articles***


1. Rane S, Das R, Ranganathan V, Prabhu S, Das A, Mattoo H, Durdik JM, George A, Rath S, Bal V (2014) Peripheral residence of naïve CD4 T cells induces MHC class II-dependent alterations in phenotype and function. *BMC Biol* **12**: 106-125.
2. Tufail S, Owais M, Kazmi S, Balyan R, Khalsa JK, Faisal SM, Sherwani MA, Gatoo MA, Umar MS, Zubair S (2015) Amyloid form of ovalbumin evokes native antigen-specific immune response in the host: prospective immuno-prophylactic potential. *J Biol Chem* **290**: 4131-4148.
3. Rathore DK, Nair D, Raza S, Saini, Singh S, Kumar A, Tripathi R, Ramji S, Batra A, Aggarwal KC, Chellani HK, Arya S, Bhatla N, Paul VK, Aggarwal R, Agarwal N, Mehta U, Sopory S, Natchu UC, Bhatnagar S, Bal V, Rath S, Wadhwa N (2015) Underweight full-term Indian neonates show differences in umbilical cord blood leukocyte phenotype: a cross-sectional study. PLOS One **10**:e0123589.




# Prafullakumar B. Tailor

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

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Transforming Growth Factor-β (TGF-β) super family controls many cellular events, immune functions and tumor metastasis. TGF-β signaling shows important roles in Dendritic Cell (DC) development. In DCs, TGF-β signalling induces expression of *Id2*, a member of helix-loop-helix (HLH) group of transcription factors, which are antagonists of activator class of HLH members. Our recent study demonstrated that *Irf8* expression leads to *Id2* gene induction in DCs. *Irf8 was* identified as a direct target of TGF-β signalling during DC development. Hence, we hypothesized that TGF-β signalling induced *Id2* gene expression in DCs that may be mediated by *Irf8.* To address our hypothesis, we started *in vitro* experiments by TGF-β treatment of BMDC cultures, which led to efficient induction of TGF-β induced genes e.g. *Tgfbi*, *Pdgfb* and *Smad7* and this induction was inhibited by SB431542, an inhibitor of TGF-β type I receptor. Transcripts of *Irf8*, *Irf4* and *Id2* genes that play critical role in DC development remained at comparable levels in cultures treated with TGF-β in presence or absence of SB431542. IRF8 protein levels were also comparable in presence of SB431542, while it efficiently inhibited SMAD2 phosphorylation. Our results, thus suggest that *Irf8* gene induction or protein levels in murine BMDC development may not be regulated by TGF-β signalling. To confirm our observations, we further analyzed BMDC development from bone marrow cells in continuous presence of SB431542. TGF-β signalling inhibitor was active until harvesting on day 9 as seen by inhibition of SMAD2 phosphorylation upon TGF-β stimulation, though development of pDCs and $CD8\alpha^+$ DCs were comparable. This result suggests that TGF-β signaling may not be essential for development of pDC or $CD8\alpha^+$ DC, DC subtypes that are essentially dependent on *Irf8* expression. We performed *in vitro* experiments by blocking TGF-β signalling of developed BMDCs or during culture of bone marrow cells. Hence, to address our

observations, we analyzed the DC development in transgenic mice model expressing dominant negative form of TGFBR2 under CD11c promoter (Itgax-dnTGFBR2 represented as TG mice henceforth). Representations from pDC and $CD8\alpha^+$ DC populations from *in vitro* BMDC cultures were comparable between control mice (WT) and TG mice. $CD8\alpha^+$ DC specific *Irf8*, *Batf3*, *Id2* transcripts along with pDC specific *Tcf4* and *SpiB* and $CD4^+$ DC specific *Irf4* and *Irf2* transcripts were also expressed at comparable levels in WT and TG BMDCs. Our data from the TG mice are in agreement with the recently reported phenotype from CD11c specific deletion of TGFβRII, wherein multi organ autoimmune inflammation and spontaneous activation of T and B cells led to death at 3-4 months. Here, we report that in adult TG mice homeostatic development of major splenic DC subtypes were unaffected. Our results differ from the reported study with human cord blood based culture system in terms of dependency of *Irf8* on the TGF-β signaling during DC development. This may be due to basal differences in the mouse adult bone marrow precursors *vis* a *vis* human cord blood precursors or DCs from cord blood cells were cultured using two step method with cocktail of cytokines whereas our study is based on FL-DC culture system, a bona-fide representative of diverse DC subtypes from mouse spleen. We support our observations by study from adult TG mice and from CD11c targeted TGFBR2 deleted mice. Thus, we conclusively demonstrate that TGF-β signalling may not play decisive role in controlling *Irf8* dependent DC development.

**Publication**

***Original peer reviewed article***

1. Bradfute SB, Anthony SM, Stuthman KS, Ayithan N, Tailor P, Shaia CI, Bray M, Ozato K, Bavari S (2015) Mechanisms of immunity in post-e vaccination against Ebola virus infection. *PLoS One* **10**: e 0118434.



# Vineeta Bal

## Biology of T lymphocytes

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**Theme of research**

Two major areas are under investigation. Firstly, role of infection and inflammation in immune response and secondly studies in T cell fate decisions. The first area has two components, one focussing on mechanisms regulating podocyte mediated barrier function in the kidney; and the second part on the role of T cells in Japanese encephalitis infection. In the second area CD4 T cell aging, survival, proliferation and differentiation are analysed in different systems.

**Objectives**

1. To study mechanisms associated with renal dysfunction and proteinuria.
2. To study the role of T cells in Japanese encephalitis infection in mouse model.
3. To study the Th1/Th2 differentiation fate of CD4 T cells ex vivo and in vitro.
4. To characterise the effects of in vivo aging on CD4 T cell function.

**A. To study mechanisms associated with renal dysfunction and proteinuria.**

In the mouse model of nephrotic syndrome, we primarily used LPS, a TLR4 ligand to induce albuminuria. Since TLR expression on BM-derived cells and CD80 expression/induction on podocytes appears critical we looked for the connecting link, a possible soluble mediator to connect these two events. Serum cytokine

levels following LPS injection showed high serum levels of IL-6, TNFα and IL-10 with different time kinetics. This was associated with increase in serum CD80 levels as well. While CD80-/- mice showed up regulation of serum cytokines to the same extent, there was no CD80 detectable in serum. Based on published reports we speculated that TNFα might be the soluble factor responsible for albuminuria. Direct injection of TNFα in B6 mice led to albuminuria whereas CD80-/- mice were resistant. We also used TNFα antagonist Etanercept to block in vivo TNFα action. Injection of LPS along with Etanercept significantly inhibited the extent of albuminuria and CD80-uria in B6 mice, confirming the role of TNFα in the pathology.

We also identified the potential signalling intermediates responsible for TNFα mediated up regulation of CD80 on podocytes in vitro. We found that p38 MAPK, MEK/Erk signalling was involved which possibly led to NFκB-mediated transcriptional up regulation of CD80 in the nucleus. We confirmed that TNFα mediated signalling in podocytes leads not only to induction of CD80 mRNA but also protein expression.

IL-10 is an anti-inflammatory cytokine with a dominant role in alternate activation of macrophages. IL-10-/- mice show a hyperinflammatory phenotype. LPS injection also leads to up regulation of serum IL-10 levels. We therefore examined the role of IL-10 in our model. IL-10-/- mice were more sensitive to TLR mediated up regulation of serum TNFα as well as albuminuria and CD80-uria. Since we have shown that chitohexaose (Chx) can inhibit LPS mediated albuminuria, and it is an activator of alternate activation pathway of macrophages, we further looked for its role in the pathology. Chx injection along with LPS led to lowering of serum TNFα level and enhancement of IL-10 level, suggesting that it may be acting through IL-10 induction. This was confirmed when Chx was injected along with LPS in IL-10-/- mice. While this treatment decreased albuminuria and CD80-uria in B6 mice, there was no effect on albuminuria or CD80-uria in IL-10-/- mice, implying that IL-10 is necessary for Chx action. Both LPS and Chx use TLR4 as the receptor and hence inhibition of LPS-mediated induction of albuminuria and CD80-uria could be explained by competitive inhibition of LPS action. We also confirmed it by using C3H/HeJ and C3H/OuJ mice, where C3H/HeJ mice are resistant to Chx mediated effects. However, interestingly, when poly (I: C), a TLR3 ligand, was used to induce albuminuria, Chx treatment of mice could still inhibit albuminuria. These data suggest that Chx mediated alternate activation of macrophages leading to IL-10 production can have inhibitory effects on cells activated by other signals.

Together, our data propose a pathway where TLR-mediated inflammation-induced TNFα secretion by BM-derived cells leads to p38 MAP kinase, MEK/Erk and NFκB signalling dependent CD80 induction on podocytes with consequent proteinuria. Etanercept blocks this pathogenetic process by inhibiting TNFα-mediated CD80 induction on podocytes, and Chx blocks it by TLR4-mediated M2 macrophage activation leading to IL-10 production negatively regulating TNFα production, thus identifying potential therapies for use in minimal change NS.

**B. To study the role of T cells in Japanese encephalitis infection in mouse model.**

We had shown by adoptive transfer approach that CD8 T cells are necessary to provide protection from JE infection in our mouse model. We next characterised role for CD4 T cells. Neutralising antibodies (Abs) offers protection from JE infection and hence it is expected that JE-specific CD4 T cells would be necessary. First, we estimated JE-specific antibodies in WT and TCRβ-/- mice. While JE-specific IgM Abs were detectable in both strains of mice on day 5 and day 12 post-infection, IgG Abs were detectable only on day 12 post-infection in WT mice, not in TCRβ-/- mice, as expected. Next, MHCII-/- mice were infected in parallel with WT mice and mortality observed. MHCII-/- mice survived as well as WT mice despite near absence of CD4 T cells in the periphery. We also examined whether any of the cytokines commonly associated with effectors CD4 T cell function influence the outcome of JE infection. Thus, IL-4-/-, IL-10-/- and IFNγ-/- mice were infected with JE and mortality compared with WT mice. To our surprise, these mice were as resistant to JE infection as WT mice. Transfer of T cells from these mice to TCRβ-/- mice prior to infection also offered protection comparable to that observed with transfer of WT T cells. Together, these data show that while CD4 T cells are required for Ab production in JE infection, during primary exposure to JE CD4 T cells and Abs may not be critically important, unlike CD8 T cells.

**C. To study the Th1/Th2 differentiation fate of CD4 T cells *ex vivo* and *in vitro*.**

In our efforts to understand how memory cells generated *in vivo* over the lifetime of the murine or human host behave in terms of cytokine production and transcriptional regulation, we have been comparing *ex vivo* CD4 memory cell functions with naïve CD4 T cells activated *in vitro*. We had observed that polyclonal memory population *in vivo* is capable of producing either Th1 or Th2 type of cytokines despite co-expression of transcription factors t-bet and gata-3. We confirmed these observations using dual colour Elispot assay for *ex vivo* memory cells from C57Bl.6 as well as Balb.b mice, where we found near absence of T cells capable of producing both IFNγ and IL-4. We extended these findings in two different ways. Firstly, we examined *ex vivo* memory CD4 T cells from an additional mouse strain FVB and used memory CD4 cells from aged B6 mice. Both of them also showed near absence of dual cytokine secreting T cells. Earlier we had reported that naïve CD4 T cells activated in non-polarising conditions *in vitro* also show co-expression of transcription factors but absence of dual expression of cytokines. These findings were extended using naïve CD4 T cells from TCR transgenic mice. Antigen-specific activation of OT-II cells from Balb.b mice *in vitro* also resulted in co-expression of transcription factors but absence of dual expression of cytokines.

**D. To characterise the effects of *in vivo* aging on CD4 T cell function.**

After showing that naïve CD4 T cells expressing lower levels of CD4 are individually older cells by extensive functional and phenotypic characterisation, and identifying DUSP6-miR181a mediated signalling as a key event, we looked for potential differences in early signalling events post-activation. We did not find any differences in calcium flux or phosphorylation of proximal signalling kinases such as Zap70 or Lck between naïve CD4lo and CD4hi cells.

**Publications**

***Original peer reviewed articles***


1. Rane S, Das R, Ranganathan V, Prabhu S, Das A, Mattoo H, Durdik JM*, George A*, Rath S*, Bal V* (2014) Peripheral residence of naïve CD4 T cells induces MHC class II-dependent alterations in phenotype and function. *BMC Biology* **12:** 106.
2. Rathore DK, Nair D, Raza S, Saini S, Singh R, Kumar A, Tripathi R, Ramji S, Batra A, Aggarwal KC, Chellani HK, Arya S, Bhatla N, Paul VK, Aggarwal R, Agarwal N, Mehta U, Sopory S, Natchu UCM, Bhatnagar S, Bal V, Rath S and Wadhwa N (2015). Underweight full-term Indian neonates show differences in umbilical cord blood leukocyte phenotype: a cross-sectional study. *PLoS One* **10**: e 0123589.


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# Agam Prasad Singh

## *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. Those could prove good targets for drug development. Parasite kinases, phosphatases etc. targeted to hepatocytes are likely candidates. The host processes affected by them could also be target for intervention. Basic theme is to identify, new parasite molecules that affect the host cellular processes, and possible intervention strategies. We use reverse genetics tool to characterize the parasite proteins with regard to its function.

***Plasmodium berghei* liverstage specific protein SLTRiP, *Immunization provides protection against sporozoite challenge and protection* is T cell dependent*:***

Mice immunized with purified SLTRiP protein yielded a very high antibody titer of $5x10^6$ after second booster immunization. The specificity of mouse anti-SLTRiP antibody was determined by western blot. Mouse anti-SLTRiP antibody specifically recognizes SLTRiP in *P.berghei* wild type sporozoites lysate but not in SLTRiP-KO sporozoites and *P.berghei* (wild type) blood stage parasite lysate showing a very high degree of specificity. Absence of SLTRiP antibody cross-reactivity, with blood stage parasite proteins highlight that SLTRiP is different from other tryptophan rich proteins expressed during blood stages. To test the vaccine potential of SLTRiP protein, BALB/c mice were immunized with purified SLTRiP protein and challenged with $1x10^4$ wild type sporozoites. In control and SLTRiP, immunized group, blood stage parasites were detected on $3^{rd}$ and $7^{th}$ days respectively, highlighting that SLTRiP gives strong but partial protection against sporozoite challenge. Further, the result was verified by checking parasite burden in the livers of immunized and control mice 48 hours post challenge with sporozoites. Results show that the parasite load

in the liver of SLTRiP immunized mice was four logs (10,000 times) less as compared to the control group of mice.

To ascertain the contribution of cell mediated immunity, we immunized RAG-1$^{null}$ mice with SLTRiP and measured the loss of protection compared to genotype matched controls (C57BL/6). RAG-1$^{null}$ mice which lacks both B and T cells, showed a 100-fold loss in protection [out of 1000 fold protection in matched wild type control] indicating T cells play major role in protection since antibodies give minimal protection as determined by passive transfer of serum to the naïve mice followed by sporozoite challenge. Using another method of sporozoite antibody neutralization assay [SNA], we tested the contribution of anti-SLTRiP antibodies in protection. To our surprise, at 1:5 dilutions we observed only two-fold less infection compared to control, indicating minimal role of antibodies in protection.

**New drug screening against liver-stage parasites:**

Andrographolide a diterpene lactone purified from methanolic fraction of the plant *Andrographis paniculata* have been shown to have antimalarial activity against the blood stage of *Plasmodium*. We tested Andrographolide against the liverstage of *Plasmodium*. We found that after drug treatment [per oral] at 5mg/mouse x3 days, followed by sporozoite challenge, the Andrographolide treated mice led to a 3-day delay in the pre patent period thus causing a 1000 fold reduction in initial parasite load with respect to the control.

**Publications**

***Original peer-reviewed articles***


1. Patel TK, Anand R, Singh AP**,** Shankar J, Tiwary BN (2014) Evaluation of Aflatoxin B1 biosynthesis in *A. flavus* isolates from central India and identification of atoxigenic isolates. *Biotech Bioproc Engn* **19**:1105-1113.
2. Kaiser K, Camargo N, Kappe SHI, Singh AP (2014) *Plasmodium* axenically developed exo-erythrocytic forms immunization confer strong protection against infectious sporozoite challenge. *Austin J Vaccines Immunother* **1**: 6.
3. Anand R, Shanka, J, Tiwary BN, Singh AP (2015) *Aspergillus flavus* induces granulomatous cerebral aspergillosis in mice with display of distinct cytokine profile. *Cytokine* **72:** 166-172.


***Reviews/Proceedings***


1. Jaijyan DK, Singh H, Singh, AP (2014) Malaria liver stage parasites strategies for immune evasion and host modulation: Implication for vaccine design. *Austin J Vaccines Immunother* **1**: 4.
2. Dalai SK, Yadav N, Patidar M, Patel H, Singh AP (2015) Liver-stage specific response among endemic populations: diet and immunity. *Front Immunol* **6**:125



# Akhil C. Banerjea

## HIV-1 Pathogenesis by Tat/Rev and accessory proteins

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HIV-1 has evolved novel strategies to escape or degrade host restriction factors, which ensures virus growth. The two major regulatory proteins of HIV-1 are Tat and Rev. They are responsible for enhanced gene expression. Nothing was known about the intracellular regulation of the two proteins at the post-translational level. We found a novel activity of Rev Protein in causing reduction of Tat protein via NQO1. HIV-1 accessory proteins (Nef, Vpu, Vif, and Vpr) have profound effects on HIV-1 pathogenesis. While Nef degrades p53, Vpu stabilizes p53 in a Beta-TrCP dependent manner. This stabilization correlates with the extent of apoptosis in human T-cells. Natural Vpu variants were genetically and functionally characterized and a natural B-TrCP mutant was described that was functionally compromised. Thus, p53 levels are down in the early stages but high during the late stage, a situation favourable for the virus. We recently reported that Vpr is a global inhibitor of host cell ubiquitination and that it redirects the ubiquitination machinery for selectively destroying HIV-1 host-restriction factors. The role of P53-sensitive miRNA, 34a, its target PNUTS, was investigated in detail during HIV-1 infection. MicroRNAs (miRNAs) are a class of small non-coding RNAs that constitute an essential and evolutionarily conserved

mechanism for post-transcriptional gene regulation. Recently, several viral mechanisms for escaping antiviral RNAi has been discovered which includes suppression of RNAi, mutational escape from RNAi and modulation of the cell's microRNA (miRNA) profile. Similar to plant and insect viruses, several mammalian viruses encoding RNAi silencing suppressors (RSS) have been described. We have explored in detail whether RNAi-mediated effects could influence HIV-1 infection-specific outcomes. Arginine Rich Motif (ARM) of HIV-1 Tat and Rev are extensively studied linear motifs (LMs) that are essential for HIV-1 gene expression. Our work explores the role of HIV-1 ARM as a uniquely evolved viral motif to combat Dicer-dependent RNAi. We reported that Rev is endowed with powerful RNAi-suppressor function. We also observed that NS4B of Dengue virus is a powerful RNAi-suppressor and in this case, ARM motif is sufficient to confer that property. Our model argues that HIV-1 suppresses the processing of siRNAs through inhibition of Dicer while at the same time manipulates the RNAi machinery to process miRNA involved in HIV-1 replication from Dicer independent pathways.

**Publication**

***Original peer reviewed article***

1. Arora S, Verma S, Banerjea AC (2014) HIV-1 Vpr redirects host ubiquitination pathway. *J. Virol.* **88**: 9141-9152.



# Anna George

## Study of mucosal immune responses

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Over the current reporting year, we found that CD40 ligation on naïve B cells leads to a reduction in Blmip-1 transcripts within 12 h. This suggested that the transcription factor might be under active repression in B cells in vivo via B-T interaction. In support of this, we found that Blimp-1 transcripts were higher in naive B cells from CD40-/- mice as compared to cells from their wild-type (Balb/c) counterparts, and lineage specificity of the phenotype was confirmed in bone marrow chimeras. Blimp-1 transcripts were also higher in TCR$\beta^{-}\delta^{-}$ mice than in B6 wild types. Treatment of B6 mice with anti-CD40L antibody led to an increase in Blimp-1 transcripts at 72 h as compared to cells from mice treated with an isotype control. Finally, treatment of TCR$\beta^{-}\delta^{-}$ B cells with anti-CD40 (to provide the ligand that is absent in these mice) led to Blimp-1 repression but, as expected, had no effect on CD40-/- B cells. Together, the data indicate that ligation of CD40 on B cells with CD40L on T cells keeps Blimp-1 repressed in vivo. We also found that down modulation of Blimp-1 by CD40 ligation was a dominant effect. Together, the data indicate that CD40 ligation around the time of B cell activation, such as provided by interaction of antigen-specific B cells with activated T cells during immunization, can modulate B cell differentiation decisions, and suggests that CD40 may provide a 'constitutive' signal in vivo of relevance for such decisions by altering the differentiation-threshold for B cells. In support of this, we found that CD40-null B cells are more poised towards terminal differentiation and show enhanced generation of plasma cells upon stimulation.

Since the generation of plasma cells and memory cells are mutually exclusive events, and since IgM+, cells form a significant fraction of the memory B cell pool, we also tested whether the enhanced tendency to terminal differentiation in CD40-/- mice was accompanied by lower frequencies of memory B cells to environmental antigens. B cell

memory been classified into distinct subsets based on the expression of CD73, CD80 and CD273 and hence we also assessed their relative proportions in unmanipulated WT and CD40-null mice to see whether CD40 signaling through T-B interactions affected the generation of any or all of these subsets. IgM memory B cells were identified in splenocytes as $B220^{+}IgM^{+}IgD^{-}CD93^{-}CD21/35^{-}CD23^{+}$ cells and their frequency as well as the frequency of each IgM memory subset was estimated. IgM memory cells were found to be lower in the spleens of CD40-/- mice, with severe depletion of the CD73+ population.

Together, our data indicate that CD40 actively represses Blimp-1 in vivo and prevents early differentiation of activated B cells into plasma cells. This presumably supports their progression into germinal centers and the acquisition of somatic mutations before they undergo terminal differentiation, and provide a mechanistic link between memory generation and the requirement for germinal centers and T cells. They also demonstrate that a specific fraction of IgM memory is T cell dependent.

**Publication**

***Original peer reviewed article***

1. Rane S, Das R, Ranganathan V, Prabhu S, Das A, Mattoo H, Durdik JM, George A, Rath S, Bal V (2014) Peripheral residence of naïve CD4 T cells induces MHC class II-dependent alterations in phenotype and function. *BMC Biol* **12**:106.



# Ayub Qadri

## Analysis of *Salmonella Typhi*-host cell interaction

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Pathogenic *Salmonella* species produce different clinical manifestations depending upon *Salmonella* serovar and the type of host. *Salmonella Typhi* causes systemic infection, typhoid, in humans but does not establish infection in mice. On the other hand, non-typhoidal serovar *Salmonella Typhimurium* causes only self-limiting localized gastroenteritis in humans but produces a systemic infection in mice that is analogous to human typhoid. The reasons for different clinical outcomes in humans and for the host specificity exhibited by these two closely related *Salmonella* serovars are not understood. Work in our laboratory focuses on identifying differences in host-pathogen interactions that ensue during infection with *S.Typhi* versus *S.Typhimurium*, and on understanding how pathogenic *Salmonella* manipulates host immune responses in order to establish infection.

**Vi suppresses activation of Rac-1/Cdc-42 activation during *Salmonella* infection**

Our previous findings showed that *Salmonella Typhi* could employ its virulence polysaccharide, Vi, to suppress inflammatory and innate immune responses which are activated through Toll-like receptors (TLRs) during infection of intestinal epithelial cells (IECs) and mononuclear phagocytes with this pathogen. However, it is known that during infection of IECs, inflammatory responses with *Salmonella* are not only produced through ligation of membrane and endosomal TLRs but also through activation of Cdc42/Rac-1 by effectors, which are delivered by pathogenic *Salmonella* intracellularly. We therefore asked a question if engagement of membrane prohibitin with Vi would also modulate this kind of inflammatory response. The results showed that treatment with Vi abrogates activation of Rac-1 and reorganization of actin cytoskeleton in *Salmonella*-infected cells. This modulation suppresses activation of NF-κB and MAP-kinase

pathways of intracellular signalling and consequently down regulates production of neutrophil chemoattractant CXCL8 and other immune mediators. Our results provide new insights into how targeting of membrane prohibitin with Vi can inhibit antimicrobial epithelial responses and contribute to establishment of infection with *S.Typhi.*

***Caspase-1 regulates replication of Salmonella in macrophages***

Caspase-1 activation following infection with pathogenic *Salmonella* results in cell death associated with release of IL-1β and IL-18, collectively termed pyroptosis. One of the bacterial effectors that bring about caspase-1 activation is flagellin. Previous work from our laboratory showed that sensing of lysophospholipids derived from intestinal epithelial cell (IEC) or pyroptotic macrophages activates release of proinflammatory flagellin from *Salmonella* thereby revealing a novel mode of regulation of inflammatory responses during infection with this pathogen. In addition to flagellin (and *Salmonella* pathogenicity island-1 effectors) which activate caspase-1 through activation of the Nlrc4 inflammasome, *Salmonella* also brings about caspase-1 activation through activation of the Nlrp3 inflammasome by *Salmonella* pathogenicity island-2 (SPI-2) - dependent yet unidentified ligand; SPI-2 is required for the establishment of systemic infection. These observations raised a possibility that Nlrp3-dependent caspase-1 activation might occur during later stages of infection. We therefore sought to investigate if casapse-1 activation that might be triggered through SPI-2 - dependent mechanism has a role to play in bacterial replication/persistence. Interestingly, *ex vivo* treatment of splenic macrophages obtained from infected mice with specific caspase-1 inhibitor resulted in reduction in intracellular bacterial numbers in a concentration dependent manner. Conversely, treatment of these infected macrophages with casapse-1 activator increased intracellular bacterial load. These results reveal a previously unappreciated role for caspase-1 in regulating bacterial replication during establishment of infection with pathogenic *Salmonella*.

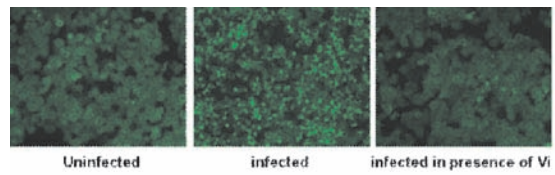



**Figure 1: Vi inhibits *Salmonella* induced actin cytoskeletal rearrangement.** Hela cells were infected with *S.Typhimurium* for 30 minutes in the absence or presence of Vi. Cells were stained with FITC-labeled Phalloidin and observed under a fluorescence microscope.

**T cell receptor - generated signals regulate innate immune responses from human T cells**

We reported previously that freshly isolated CD4 T cells from human peripheral blood secrete neutrophil chemoattractant CXCL8 upon stimulation with TLR2 and TLR5 agonists Pam3CSK4 and flagellin respectively. However, when these cells were first activated through the T cell receptor, their ability to produce CXCL8 was considerably reduced. We now show that the reduction in CXCL8 secretion is also observed during stimulation of T cells with the endogenous innate stimulus, IL-1β. Importantly, while freshly isolated CD4 T cells did not produce any IFN-γ upon incubation with Pam3CSK4 and flagellin, TCR-primed cells began to secrete IFN-γ in response to these TLR

agonists in the absence of concurrent TCR engagement. These T cells showed increased activation of p38 and JNK MAP-kinases in response to TLR stimulation, and inhibition of p38 abrogated TLR-induced IFN-γ secretion. Our results suggest that the innate immune response of human T cells might shift from a proinflammatory to an effector type following activation of these cells through the T cell receptor. These findings have significant implications for T cell-mediated antimicrobial immunity.

**Publication**

***Original peer-reviewed article***


**1.** Santhanam SK, Dutta D, Parween F, Qadri A (2014) The Virulence polysaccharide Vi released by *Salmonella typhi* targets membrane prohibitin to inhibit T cell activation. *J Infect Dis* **210**: 79-88

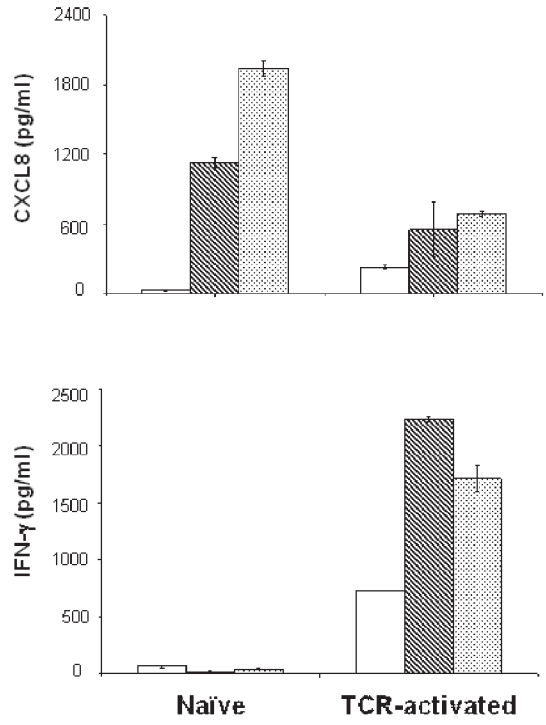




**Figure 2: T-cell receptor generated signals modulate TLR response from human T cells**. Naïve and TCR-activated human CD4 T cells isolated from peripheral blood were stimulated with TLR2 and TLR5 ligands, Pam3CSK and flagellin respectively. 24h later, CXCL8 and IFN-γ levels were determined in the supernatants.



# Devinder Sehgal

## Microbial interface biology and associated host immune response

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The theme of research is to decipher how *Streptococcus pneumoniae* (pneumococcus) causes disease and what interventions are needed to stop this from happening. The research focuses 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 proteins.

**Identification and functional characterization of secreted nuclease from *S. pneumoniae***

The neutrophils clear pneumococci by phagocytizing the bacteria followed by killing. In addition, they release neutrophil extracellular traps (NETs), which are a framework of chromatin, histones and antimicrobial proteins. NETs entrap pneumococci and prevent their dissemination in the host. The endonuclease EndA from *S. pneumoniae* has been reported to be involved in degrading NETs. Published data indicates the presence of additional nuclease(s). We are interested in identifying and characterizing these novel nuclease(s). To test whether pneumococcal culture supernatant degrades NETs, neutrophils were isolated from the peritoneal cavity of mice and stimulated with phorbol 12-myristate 13-acetate to induce NET

formation. Supernatant from pneumococcal culture degraded DNA present in NETs in a dose dependent manner as visualized by confocal microscopy. Furthermore, the putative DNase(s), in-gel activity assay was performed with the pneumococcal culture supernatant. We observed 3 bands with DNase activity. Mass spectrometric analysis identified SPD_1788 as a probable candidate. The gene encoding SPD_1788 was PCR amplified from *S. pneumoniae* D39 genome, cloned and expressed in *E. coli*. SPD_1788 was purified to homogeneity from inclusion bodies. Further characterization is currently underway.

**Functional characterization of lipoproteins from *S. pneumoniae***

Lipoproteins constitute one of the most abundant classes of surface proteins of *S. pneumoniae*. Given their surface localisation, and importance in pneumococcal fitness and virulence we focussed our study on the functional characterization of pneumococcal lipoproteins. We used a model murine alveolar macrophage cell line MH-S for our study. Alveolar macrophages from C57BL/6 mice were stimulated with Triton X-114 extract from the wildtype and *lgt* deficient *S. pneumoniae*. We observed a significant increase in the production of proinflammatory cytokines in the presence of Triton X-114 extract from wildtype *S. pneumoniae*. The amount of these cytokines produced in presence of Triton X-114 extract from the *lgt* deficient strain was comparable to the untreated control. The amount of nitric oxide (NO) produced by *ex vivo* alveolar macrophages from C57BL/6 mice was significantly enhanced in presence of Triton X-114 extract from wildtype *S. pneumoniae* compared to the corresponding extract from the *lgt* deficient strain. Incubation of MH-S cells with Triton X-114 extract from wildtype *S. pneumoniae* resulted in the upregulation of inducible nitric oxide synthase (iNOS). There was no induction of iNOS in control samples treated with Triton X-114 extract from the *lgt* deficient *S. pneumoniae*. Next, we stimulated MH-S cells with Triton X-114 extract from wildtype and *lgt* deficient *S. pneumoniae* in presence of anti-TLR2 blocking antibody. We observed significant abrogation in the production of TNF-α, IL-6 and IL-12 as well as NO in wildtype Triton X-114 extracts stimulated samples in presence of anti-TLR2 blocking antibody. This led us to conclude that recognition of pneumococcal lipoproteins by TLR2 is important for modulation of alveolar macrophage function by *S. pneumoniae*. We observed that lipoproteins activated MAPKs and NF-κB pathways. Using signalling pathway specific inhibitors, we found that both ERK and JNK governed production of proinflammatory cytokines IL-6 and TNF-α and NO production was largely influenced by JNK pathway.

**Publications**

***Original peer-reviewed articles***


1. Anish C, Upadhyay AK, Sehgal D, Panda AK (2014) Influences of process and formulation parameters on powder flow properties and immunogenicity of spray dried polymer particles entrapping recombinant Pneumococcal surface protein A. *Int J Pharm* **466**:198-210.
2. Khan N, Qadri RA, Sehgal D* (2015) Correlation between *in vitro* complement deposition and passive mouse protection of anti-Pneumococcal surface protein A monoclonal antibodies. *Clin Vaccine Immunol* **22**:99-107.
3. Saxena S, Khan N, Dehinwal R, Kumar A, Sehgal D* (2015) Conserved surface accessible nucleoside ABC transporter component SP0845 is essential for pneumococcal virulence and confers protection *in vivo*. *PLoS One* **10**:e 0118154.



# Amulya K. Panda

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

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**Objectives**

The objective of the research project in the laboratory is to evaluate polymeric particle based delivery system for improved immunogenicity of different antigens such as Tetanus Toxoid (TT), Hepatitis B surface antigen (HBsAg), viral and carbohydrate (Vi polysaccharide and *S. 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. Progress achieved in the above two areas are described below.

**A. Immune response from polymeric particles entrapping antigens**

Polymeric particles entrapping protein/carbohydrate antigens are used in the laboratory to improve their immunogenicity. Different sized PLA particle entrapping Vi polysaccharides were used for immunization to delineate the size dependency of antibody response. It was observed that unlike protein antigens, Vi polysaccharides entrapped in nanoparticles induces better antibody response than that observed with microparticles. As nanoparticle, entrapped Vi

polysaccharide was eliciting memory antibody response and promoting antibody isotype switching, it was of interest to compare the results with conjugate vaccine. Vi polysaccharide was conjugated to flagellin using ADH linker. It was hypothesized that conjugation of polysaccharides with homologous protein antigens (Vi polysaccharide and flagellin from same pathogenic bacteria) could be more protective, as antibodies against both antigens can work synergistically as compare to single antigen. The conjugates were purified and characterized in detail. Immunogenicity evaluations of the conjugates both in free and in nanoparticulate form are under way. The results will provide information whether plain polysaccharide entrapped in nanoparticles are comparable or better than that of conjugate vaccines in terms of generating antibody response.

**B. Solubilization and refolding of inclusion body proteins**

Mild solubilization of inclusion body protein without using high concentration of chaotropes is being extensively used now a day for high throughput recovery of bioactive proteins. We have been continuously exploring the use of organic solvent for solubilization and refolding of inclusion body proteins. It was observed that inclusion body aggregates can be solubilized using 50 % trifluroethanol (TFE). Use of urea (3 M) helped in better solubilization of inclusion body aggregates. Human growth hormone inclusion bodies were successfully solubilized using TFE along with 3 M urea. The proteins were refolded into native conformation. This provides a new way of inclusion body solubilization and have been tested for inclusion bodies of different proteins.

Studies are being conducted in the laboratory for understanding amyloid aggregation of globular protein. Ovalbumin was used as a model protein and it was observed that by heating at 80 $^{o}$C, the protein aggregates into amyloid fibrils. Further, it was observed that in presence of organic solvent such as 5% TFE or n-propanol, ovalbumin aggregates into amyloid fibrils. The presence of organic solvents (TFE or Propanol) increased the rate of aggregate formation. We are currently studying how amyloid aggregates are being formed at physiological conditions.

**Publications**

***Original peer-reviewed articles***


1. Anish C, Upadhyay AK, Sehgal D, Panda AK (2014) Influences of process and formulation parameters on powder flow properties and immunogenicity of spray dried polymer particles entrapping recombinant pneumococcal surface protein A. *Int J Pharm* **466**: 198-210.
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1. Singh A, Upadhyay V, Panda AK (2015) Solubilization and refolding of inclusion body proteins. Insoluble Proteins. *Methods Mol Biol* **1258:** 283-291.

## Patent

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# Rahul Pal

## Disorders of proliferation: Analysis of novel pathways and targets

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### Theme of research

The lab focuses on specific instances of proliferative aberrance.

Human chorionic gonadotropin (hCG), critical for the sustenance of pregnancy, is also secreted by a variety of cancers and is associated with poor patient prognosis. The molecular mediator by which hCG influences tumor progression is being elucidated.

Systemic Lupus Erythematosus (SLE) is the prototypical non-organ specific autoimmune disease. Since impairment in the uptake of apoptotic cells results in lupus-like symptoms in mice, the influence of apoptotic debris on innate and adaptive immune responses is being investigated. In addition, whether the release of sequestered hemoglobin in an autoimmune environment constitutes a critical inflammatory trigger is being looked at.

**A. hCG and tumorigenesis**

Whether transgenically-expressed βhCG influences the growth of both endogenous and implanted tumors is being evaluated. Increased serum prolactin, weight gain, ovarian hyperplasia and pituitary adenomas characterize transgenic (TG) female C57BL/6 x FVB$^{\beta hCG/-}$ F1 mice. Higher tumor incidence and tumor volumes are observed when syngeneic lung tumor cells are implanted in such mice; tumors contain markedly higher levels of transcripts for IL-6, KC, TNFα, VEGF, MMP-2, MMP-9, Bcl-2, Bcl-xl, survivin and versican. While levels of TGFβ are higher in the sera of TG animals, KC is heightened in TG animals subsequent to tumor development. βhCG/CG therefore up-modulates molecules known to play critical roles in tumor progression.

**B. Systemic autoimmunity**

***The immunobiology of hemoglobin (Hb)***

Isotype-switched autoantibodies to Hb are detected in sera derived from SLE patients and lupus-prone mice. Such mice express higher anti-Hb B cell precursor frequencies and Hb immunization preferentially enhances autoantibody sequestration in the kidneys; split, thickened basement membranes and the deposition of complement 3 is observed in glomerulii, and the onset of nephritis is accelerated. These results establish Hb as an autoantigen and a pathogenesis-inducing immunogen in systemic autoimmunity.

Ferric Hb induces the functional maturation of bone marrow-derived dendritic cells (BMDCs) derived from lupus-prone mice. The inhibition of Stat3 most effectively down-modulates ferric Hb-induced phenotypic changes, a finding that deserves further attention.

Previous studies in our lab have described the association of ferric Hb with several autoantigens. The expression of co-stimulatory markers on BMDCs derived from lupus-prone mice is preferentially enhanced upon the addition of ferric Hb along with (autoantigen-containing) apoptotic blebs; inflammatory cytokines are secreted at significantly higher concentrations. The interaction of Hb with self-antigens released during apoptosis may therefore result in enhanced inflammatory effects. The fact that lupus-prone mice express higher circulating levels of Hb makes these observations relevant to disease etiology.

***The influence of apoptotic blebs and apoptotic cell-specific antibodies on immune function***

Aberrant apoptosis is a hallmark of SLE. Apoptotic blebs more effectively induce the maturation of BMDCs derived from lupus-prone mice, as do monoclonal antibodies, which specifically bind apoptotic cells. Upon immunization with such antibodies in syngeneic, autoimmune-prone mice, hyper-gammaglobulinemia, expansion of the autoantibody repertoire and severe glomeruosclerosis are observed. Innate and adaptive immune responses to the products of cell death can therefore perpetuate autoimmune pathology.



# Rajni Rani

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

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**Genetic basis of Type 1 diabetes and vitiligo**

We analyzed the data of 239 T1D subjects and 752 normal healthy controls from North India for *LMP2 codon 60 G/A* (R/H), *LMP7 codon 49 of C/A* (Q/K) and *LMP7 Intron 6 G/T* polymorphism and *HLA* class-II alleles. Significant increase of *LMP2* and *LMP7* haplotypes *GCG* and *ACT* in T1D ($p < 5.9 \times 10^{-13}$, $p < 1.9 \times 10^{-8}$ respectively) suggests their role in auto-antigen processing for presentation by MHC class-I molecules to cytotoxic T cells in T1D.

A gain of function mutant at nucleotide position *1858 C>T* in *PTPN22* (*Protein tyrosine phosphatase non-receptor, type 22*) gene was studied in 250 T1D patients and 480 healthy controls. PTPN22 is a negative regulator of T cell signaling. Significant association of the *T* allele and heterozygous genotype *1858CT* was observed in T1D independent of the predisposing *Human leukocyte antigen* (*HLA*) alleles *DRB1*03:01*, *DRB1*04:01*, *DRB1*04:05* in North Indian patients, suggesting their integrated roles in manifestation of T1D.
We have also studied genotype, allele and haplotype frequencies *LMP2* and *LMP7* in 1320 vitiligo patients and 752 healthy controls. *TAP1* and *TAP2* SNPs have been studied in 746 vitiligo patients and 748 healthy controls. In *LMP 7*

*exon 2* allele, *C* and homozygous *CC* (QQ) were significantly reduced in all vitiligo, generalized and localized vitiligo patients. For *TAP1 intron 5 C/T*, *exon 10 G/A* (D63G), *TAP2 exon 5 A/G* (V379I) and *TAP2 exon 11 A/G* (T665A) were studied. Significant increase in heterozygous *GA* in *TAP1 exon 10* and *AG in TAP2 exon 11* with relative risk of 1.9 and 4.1 were observed in vitiligo compared to healthy controls. Further analysis with type of vitiligo, age at onset and gender bias is going on.

**Mesenchymal stem cell (MSC) treatment of non-obese diabetic**

To differentiate MSCs into insulin producing cells (IPCs), they were sorted based on their surface markers CD29, CD73 and CD44 using FACS sorter and then differentiated into IPCs using stage specific differentiation protocol, i.e. mesodermal lineage to definitive endodermal lineage followed by pancreatic endodermal lineage and finally pancreatic hormone secreting cells and were stained for insulin using Immunocytochemistry which shows islet cell like cluster as shown in the figure 1. These IPCs along with cytokine treated MSCs are being tested in NOD mice for their potential to secrete insulin in-vivo and inhibit autoimmune responses respectively, to take care of diabetes in them.

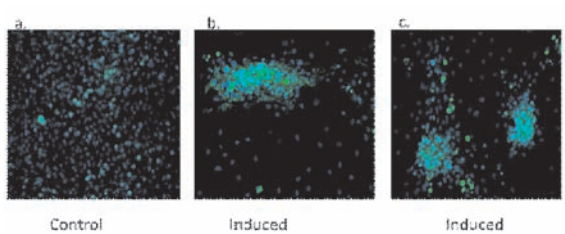



**Figure 1.** MSCs were differentiated into insulin producing cells. Control uninduced (a) and induced cells (b and c) are shown by immunocytochemistry for insulin, islet-like clusters were observed in the induced MSCs.

***Comparison of lesional vs non-lesional skin of vitiligo***

The literature has implicated increased oxidative stress in the etiology of depigmented patches in vitiligo and mitochondria have been implicated in generation of ROS. Majority of ROS is produced by oxidative phosphorylation (OXPHOS) or mitochondrial respiration. We observed higher expression of genes involved in OXPHOS in lesional and compared to non-lesional skin of vitiligo patients. The implications of this upregulation of OXPHOS in vitiligo are being investigated.

**Publications**

***Original peer-reviewed articles***

1. Saida B, Dani P, Patnaik N, Agrawal B, Rajaratna T, Jaiswal A, Singh AK, Rani R (2014) Haplotypes of polymorphic antigen processing genes for low molecular mass polypeptides *(LMP2* and *LMP7)* are strongly associated with Type 1 diabetes in North India. *J Diabetes Metab* **5**:451.
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1. Rani R, Singh A (2014) Functional implications of MHC associations in autoimmune diseases with special reference to Type1 diabetes, Vitiligo and Hypoparathyroidism. Accepted for publication in the book "HLA & Associated Human Diseases", ISBN 980-953-307-1107-5. In Tech Open Access Publishers, Janeza Trdine 9, Rijeka, Croatia



# Sangeeta Bhaskar

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

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This study aims 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. Evaluation of immunotherapeutic efficacy of *MIP* along with chemotherapy in animal infection models.

Another objective is to evaluate immunotherapeutic activity of *MIP* in mouse syngeneic tumor model and simultaneous study of underlying mechanism of *MIP.*

**A. Protective efficacy of MIP against tuberculosis and mechanistic insights as compared to BCG**

***MIP induces dendritic cell activation, survival and Th1/Th17 polarization potential in TLR-dependent manner***

Dendritic cells play a critical role in activation of antitumor and antimycobacterial immune response. The effect of MIP on the behavior of dendritic cells and the underlying mechanisms were investigated. MIP leads to significant production of IL-12p40, IL-10 and TNF-α by dendritic cells and expression of costimulatory molecules CD40, CD80 and CD86 was upregulated.

MIP increased dendritic cell survival by inhibiting apoptosis. Consistently, higher expression of anti-apoptotic proteins Bcl-2 and Bcl-xl was observed in MIP-stimulated dendritic cells. Cytokines produced by naïve T cells co-cultured with MIP-stimulated dendritic cells

showed that MIP promotes Th1/Th17 polarization potential in dendritic cells. Response to MIP was lost in MyD88-deficient dendritic cells. Further studies revealed that TLR2 and TLR9 are involved in dendritic cell activation by MIP-live, whereas MIP-killed activates the DCs through TLR2. MIP in live and killed form was evaluated for macrophage activation and compared with BCG. Using pharmacological inhibitors and knock out mice strains, major role of TLR2 was observed in the activation of macrophages by MIP and BCG. TLR2 signaling was further delineated. With the help of HEK293 cells expressing TLR2 in homodimeric or heterodimeric form, it was observed that live MIP had distinctly higher level of TLR2/2, TLR2/1 and TLR2/6 agonist activity as compared to MIP-killed and BCG.

**B. Efficacy of MIP as a booster to BCG: Immunogenicity, protection and safety study when given by aerosol route in animal models**

BCG is effective against severe form of childhood tuberculosis however; effective protection from TB in adults is still a challenge, which indicates that there is a need to boost the protective immune response against *M.tb*. However, paradoxically, multiple doses of BCG had resulted in reduced protection. It was proposed that a booster with MIP might enhance the protective immunity in animals primed with BCG.

Two groups of mice were immunized with BCG by s.c. route and after a gap of 2 months, they were given booster with MIP by aerosol/s.c. route. While one control group was immunized with only BCG without any booster, the other control group was immunized with saline. Four week after BCG immunization *M.tb* specific immune response was reduced to low level but it increased significantly after booster with MIP and was maintained at significantly high level as compared to 'only BCG' immunized group till experimental observation period. In guinea pig *M.tb* challenge experiments, significantly lower bacterial load was observed in the groups given booster with MIP as compared to 'only BCG' immunized group at 4 wk and 8 wk after challenge with *M.tb*. mRNA expression of cytokine genes IFN-γ, IL-12 and IL-2 was significantly high in the groups given booster with MIP at 4 wk after infection. Histopathological analysis of the sections of infected lungs confirmed the bacteriological findings. These results provide evidence of higher protective efficacy in the groups given MIP booster after BCG priming.

**Publications**

***Original peer-reviewed articles***


1. Kumar P, John V, Marathe S, Das G, Bhaskar S* (2015) *Mycobacterium indicus pranii* induces dendritic cell activation, survival and Th1/Th17 polarization potential in a TLR-dependent manner. *J Leukoc Biol* **97**:511-520.
2. Kumar P, Tyagi R, Das G, Bhaskar S* (2014) *Mycobacterium indicus pranii* and *Mycobacterium bovis* BCG lead to differential macrophage activation in Toll-like receptor-dependent manner. *Immunology* **143**: 258-268.


***Review/Proceedings***


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# Soumen Basak

## Fine tuning of NF-kappaB Signaling

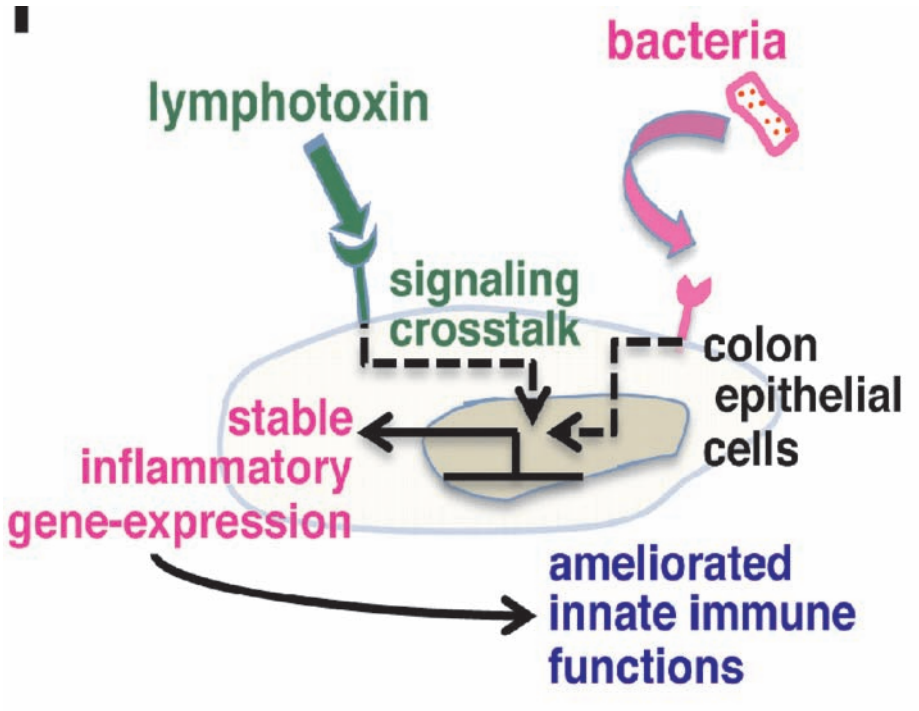
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Tissue microenvironment functions as an important determinant of the inflammatory response elicited by the resident cells. Yet, the underlying molecular mechanisms remain obscure. Our systems-level analyses identified that the pleiotropic NF-κB system connects microenvionmental cues with pathogen-derived signals in calibrating inflammatory responses in intestinal niche. First, we identified a duration code that instructs stimulus specific crosstalk between TLR4 activated canonical NF-κB pathway and lymphotoxin-β receptor (LTβR) induced non-canonical NF-κB signaling. Indeed, LTβR costimulation synergistically enhanced the late RelA/NF-κB response to TLR4 prolonging NF-κB target gene-expressions. Our mechanistic studies demonstrated thatLTβR signal targets TLR4-induced newly synthesized p100, encoded by *Nfkb2*, for processing into p52 that potently generated RelA:p52/NF-κB activity in a positive feedback loop. Finally, *Nfkb2* connected lymphotoxin signal within the intestinal niche in reinforcing inflammatory RelA/NF-κB response to Citrobacter rodentium infection, while *Nfkb2*$^{-/-}$ mice succumbed to gut infections owing to stromal defects in NF-κB activation.

**Publication**
***Original peer-reviewed article***


1. Almaden JV, Tsui R, Liu YC, Birnbaum H, Shokhirev MN, Ngo KA, Davis-Turak JC, Otero D, Basak S, Rickert RC, Hoffmann A. (2014) A Pathway Switch Directs BAFF Signaling to Distinct NFκB Transcription Factors in Maturing and Proliferating B Cells. *Cell Rep* **9**:2098-2111.




The proposed model depicting the regulatory role of *Nfkb2* in integrating lymphotoxin signals within the intestinal niche to prolong innate inflammatory NF-κB responses to gut pathogens



# Amitabha Mukhopadhyay

## Modulation of intracellular trafficking in host cells by various intracellular pathogens

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Previous studies have shown that *Salmonella* infection activates host Caspase3 and cleaves SipA into two fragments. It has been reported that C-terminal of SipA binds and localized with host actin, however, role of N-terminal fragment of SipA in the trafficking of *Salmonella* is not clearly understood. In order to understand the role N-terminal fragment of SipA, we have expressed SipA: WT, SipA$^{1-435}$ or SipA$^{436-685}$ as N-terminal 3X-FLAG fusion protein in HeLa cells. Our results have shown that SipA: WT and SipA$^{436-685}$ colocalizes with phalloidin labeled actin. Whereas, SipA$^{1-435}$ is found to be localize on distinct vesicular membrane structure. Subsequently, we have coexpressed SipA or its truncated protein along with GFP-Syntaxin8 in HeLa cells. Interestingly, we have found that SipA$^{1-435}$ significantly colocalized with Syntaxin8. In contrast, SipA: WT and SipA$^{436-685}$ are found to be colocalized with actin. Currently, we are analyzing the functional significance of SipA-Syntaxin8 interaction in *Salmonella* trafficking in host cells.

We have also reported earlier that *Leishmania* infection in macrophages triggers the expression of Rab5 in the host cells. In the reporting period, we have tried to understand the mechanism of Rab5 overexpression in macrophages by *Leishmania* infection. In the reporting period, we have shown that *Leishmania* infection specifically overproduce Rab5a in macrophages whereas levels of Rab3, Rab4 and Rab11 are not altered by *Leishmania* infection in macrophages. Moreover, real time PCR analyses have shown that overexpression of Rab5a by *Leishmania* infection is due to enhance level of Rab5a specific mRNA indicating transcriptional activation of Rab5a. Subsequently, microRNA profiling reveals that mir-494 is downregulated in *Leishmania*

infection. Interestingly, bioinformatics analysis indicates that 3′-regulatory sequence of Rab5a has mir-494 binding site. Therefore, we have cloned the 3′-regulatory sequence of Rab5a in pmirGLO (pmirGLO-3′Rab5a) luciferase containing vector. In order to determine the role of mir-494 in regulating Rab5a expression, Hela cells were co-transfected with mir-494 and pmirGLO-3′Rab5a and luciferase activity was measured after 48 hrs. Our results have shown that mir-494 significantly inhibits the transactivation of luciferase. Finally, we have shown that transfection mir-494 specifically reduces the expression of Rab5a mRNA and protein in HeLa and THP-1. Taken together, these results indicate that *Leishmania* infection inhibits the expression mir-494 in macrophages and thereby upregulated the expression of Rab5a in infected macrophages. Studies are in progress to determine functional significance of Rab5a overexpression in the trafficking of *Leishmania* in macrophages.

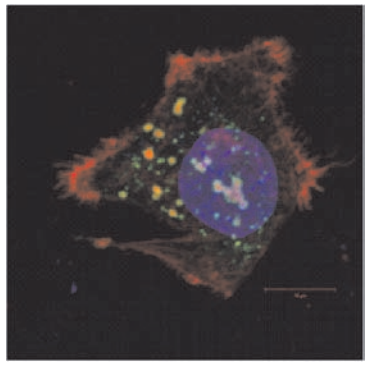


**Fig.A.**

Fig.A. Overexpression of GFP-SipA: WT in HeLa cells. Actin was stained with phalloidin.

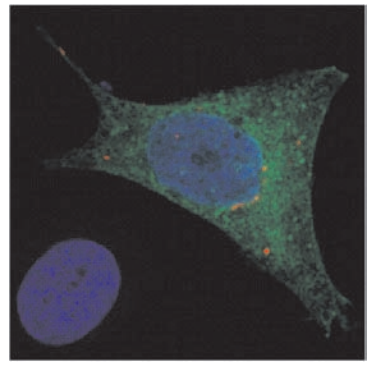


**Fig.B.**

Fig.B. Coexpression of RFP-SipA: WT and GFP-Syntaxin8 in HeLa cells.



# REPRODUCTION AND DEVELOPMENT

# REPRODUCTION AND DEVELOPEMENT


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# Subeer S. Majumdar

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

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Testicular Sertoli cells (Sc) support the division and differentiation of germ cells (Gc) upon their maturation during puberty. Recently, our lab has reported that in rats, despite sufficient levels of FSH and T during infancy (upto 9 days of age), immature Sc fail to support the robust division and differentiation of spermatogonial cells, which is only discernible during the onset of puberty, when the Sc start undergoing the functional maturation (from 12 days of age onwards). Identification of factors produced by Sc upon their maturation and their mode of actions in regulating germ cell differentiation are still missing. In order to identify the factors associated with Sc maturation, a differential evaluation of hormone responsive genes expressed by infant and pubertal rat Sertoli cell (Sc) was performed and we established the role of few of them in germ cell division and differentiation. We sequenced small RNAs from Sertoli cells of infant and pre pubertal mice in order to find out role of microRNA (miRNA) in Sertoli cell function and idiopathic infertility. We have found 532 miRNA expressing in infant Sertoli cell and 446 in pre pubertal Sertoli cells. Significantly, many microRNA clusters were differentially expressed in infant and pre pubertal Sertoli cells. We have also predicted targets of these microRNAs using miRANDA and targetscan software. Expression of the microRNA have been validated by real-time PCR. Physical interactions of miRNA and targets were validated by luciferase assay. We have generated transgenic mouse either over expressing miRNA-504 or sponge for it which

down regulates the expression of miRNA-504 in Sertoli cell specific manner.

Adult transgenic mice overexpressing sponge against miRNA-504 showed interrupted germ cell division in comparison to testis of wild type mice. On the other hand, testis of 5 days old transgenic mice over expressing miRNA-504 displayed premature germ cell differentiation and development of lumen in seminiferous tubule as compared to age matched wild type mouse testis. This provided substantial evidence suggesting significant contribution of miRNA-504 in maintaining germ cell division and differentiation.

We have initiated studying hippo signalling pathway in regulation of Sertoli cell proliferation and maturation. When YAP was inhibited using the small molecule inhibitor Verteporfin, it resulted in a dose dependent decline in the number of cells in S-phase, displaying decreased PCNA foci in cells, which is indicative of a decline in, cell proliferation. Reduced expression of Cyclin E and D1 in these treated cells supported our observation. Further studies, would involve Sertoli cell specific knockdown and over-expression of YAP to elucidate the role of YAP in Sertoli cell maturation.

We are also working on obesity-induced diabetes, which is increasing remarkably. We, in collaboration with Prof. Bhattacharya have begun testing a small molecule made by Prof. Mihir K. Choudhury from Tezpur University, on diabetic high fat fed rodents and db/db mice, to determine its feasibility as an ideal treatment for diabetes.

**Publications**

***Original peer-reviewed articles***


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2. Bhattacharya I, Basu S, Sarda K, Gautam M, Nagarajan P, Pradhan BS, Sarkar H, Devi YS, Majumdar SS (2015) Low levels of Gs and Ric8b in testicular sertoli cells may underlie restricted FSH action during infancy in primates. *Endocrinology* **156:** 1143- 1155


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# Anil Suri

## Cellular and molecular biology of human cancer

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Over the last three decades, knowledge on the molecular biology of human cancers has vastly expanded. We identified a host of genes and proteins involved in cancer development and progression. Several mechanisms at the molecular, cellular and even tissue level have been, at least partly, elucidated. In fact, cancer research has now reached a critical stage, in which the accumulated knowledge on molecular mechanisms needs to be translated into improved prevention, diagnosis, and treatment.

Head and neck squamous cell carcinoma is the sixth most common cancer in the western world. Over the last few decades little improvement has been made to increase the relatively low 5-year survival rate. This calls for novel and improved therapies. Head and neck cancer includes cancers of the mouth, nose, sinuses, salivary glands, throat, and lymph nodes in the neck. Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries. In developing nations, it

has been reported that one in four cancers in male occur in head and neck region and accounts for 30% of all cancers. Importantly, salivary gland tumor (SGT) accounts for 3-5% of total head and neck cancer and of wide histological and biological diversity. Early detection of SGT is essential for more effective clinical management, leading to improved quality of life and increased survival rate. The present study was initiated to undertake a more comprehensive analysis of SPAG9 gene and protein expression in SGT specimens in the context of clinic-pathological parameters, i.e., histo-pathological characteristics. We also investigated the humoral response against SPAG9 in various stages and histotypes of SGT patients. Our results suggest that SPAG9 may be used as a novel diagnostic biomarker for early detection of SGT thus, may be useful in better management of SGT patients.

The *SPAG9* gene expression was investigated by RT-PCR in SGT tissue along with available matched associated non-cancerous tissue (ANCT) specimens. The data revealed that 80% (82 of 102) of tumor specimens showed *SPAG9* gene expression irrespective of benign, malignant tumor, stages and various histotypes (PA: pleomorphic benign tumors, MEC: mucoepidermoid, AdCC: adenoid cystic, ACC: acinic cell, CC: clear cell, BCAC: basal cell, ANOS: adenocarcinoma not otherwise specified, PLGA: polymorphous low grade adenocarcinoma). SPAG9 protein expression was further confirmed by immunohistochemistry (IHC) in serial SGT tissue sections of benign tumors, various stages of malignant tumors and different histotypes and available matched ANCT specimens. SPAG9 protein expression was found in 80% (>10% of cells found positive for SPAG9 protein expression) of SGT specimens, whereas no expression was detected in 72 paired available matched ANCT specimens.

The circulating anti-SPAG9 antibodies were investigated in sera of 62 available SGT patients employing ELISA. Our data revealed that 68% (42 of 62) of SGT patients generated humoral response against SPAG9 protein. Interestingly, we found that these patients with anti-SPAG9 antibodies were found to be positive for SPAG9 gene and protein expression. Our data revealed that humoral response generated against SPAG9 was found in 60% (6 of 10) of benign, 90% (9 of 10) of malignant stage I, 80% (8 of 10) of stage II, 48% (11 of 23) of stage III and 89% (8 of 9) of stage IV tumors. It is important to note that 74% (14 of 19) of malignant SGT patients with positive lymph node involvement generated anti-SPAG9 antibodies as compared to 67% (22 of 33) of SGT patients with negative lymph node involvement. In addition, the patient's sera of different histotypes also revealed anti-SPAG9 antibodies which demonstrated 60% (6 of 10) of pleomorphic benign tumor patients, 72% (13 of 18) of mucoepidermoid, 63% (5 of 8) of adenoid cystic, 67% (2of 3) of acinic cell, 71% (5 of 7) of clear cell, 80% (4 of 5) of basal cell, 63% (5 of 8) of adenocarcinoma NOS and 67% (2 of 3)of polymorphous low grade adenocarcinoma. We reported the possibility of developing serum based biomarker for better cancer management of SGT patients.

**Publications**

***Original peer-reviewed articles***


1. Agarwal S, Parashar D, Gupta N, Jagadish N, Thakar A, Suri V, Kumar R, Gupta A, Ansari AS, Lohiya NK, Suri A* (2014) Sperm Associated Antigen 9 (SPAG9) Expression and Humoral Response in Benign and Malignant Salivary Gland Tumors. *Oncoimmunology* **3**:e974382.
2. Singh S, Suri A* (2014) Targeting the testis-specific heat-shock protein 70-2 (HSP70-2) reduces cellular growth, migration, and invasion in renal cell carcinoma cells. *Tumor Biol* **35**: 12695-12706.



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# Asok Mukhopadhyay

## Study on expansion and plasticity of bone marrow stem cells

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The overall theme of research in Stem Cell Biology Laboratory is in the field of Regenerative Medicine. We intended to dissect HSCs niche to elucidate its function in marrow regeneration, and further extrapolate the role of HSCs in different pathological conditions of the solid organs, and the plasticity of adult stem cells.

The main objectives of the study were:

1. Molecular control of self-renewal and engraftability of HSCs in mice.
2. To understand liver regeneration by BM-derived cells and to elucidate the mechanism of hepatic differentiation.
3. Role of BM cells in stem-ness and cancer progression.
4. Mechanistic insight in the regeneration of neurons by MSC-derived precursor cells.
5. Study of molecular interplay during fibrosis and normal regeneration of tissue.

**A. Hematopoietic stem cells niche**

We have initially assumed the role of Amphiregulin (Areg) in apoptosis of HSCs. The functional role of *Areg* gene was studied by silencing its expression in stromal cell line using shRNA technology. To assess the downstream signalling cascade of Stat5 related regulation of apoptosis in HSCs, we evaluated the expression

of pro-apoptotic and anti-apoptotic genes namely *Bad, Bax, Bcl-XL, Socs* and *Myc*. We observed an up-regulation of pro-apoptotic genes in *Areg(k/d)* stroma-dependent culture of HSCs while the results observed was inverse in wild-type stroma. We observed FasR was highly expressed in HSCs and caspase 3/7 was activated thus concluded. That the apoptosis was mainly initiated through the activation of extrinsic pathway in these calls.

**B. Plasticity in BM cells**

In acute liver injury model, we isolated BM-derived hepatocytes and performed a comprehensive transcriptional profiling to analyze the changes in gene expressions that accompanied this phenotypic conversion. We found large similarities in gene expression profile between BM-derived hepatocytes and primary hepatocytes. However, we also identified 1113 and 605 genes that were exclusively expressed in primary hepatocytes and BM-derived hepatocytes, respectively. Through gene ontology analyses, we found that even though BM-derived hepatocytes exhibited some dissimilarity in gene expression profile with respect to primary hepatocytes, there was an induction of hepatic transcriptional program with concomitant decline of hematopoiesis related program. We further investigated the epigenetic mechanisms that co-ordinate these transcriptional changes by ChIP-qPCR analysis for histone modifications. Enrichment of activating histone marks (H3K4me3 and H3K9Ac) were observed at promoters of crucial hepatic transcription factors like HNF4α, HNF1α, HNF3α, HNF3β, CEBPα, CEBPβ, HNF6 and GATA4, whereas repressive marks (H3K27me3 and H3K9me3) showed reduction at these loci after reprogramming, among which H3K27me3 mark showed prominence. In contrast to these findings, we found enrichment of repressive marks (H3K27me3 and H3K9me3) at the hematopoietic gene promoters like CD45 and GATA2 in BM-derived hepatocytes. Further work has been initiated to find out the role of demethylase enzyme JMJD3.

**C. Ovarian cancer**

Microarray gene expression profiling of human ovarian cancer samples revealed that about 1728 genes were upregulated in $EpCAM^{+}CD45^{+}$ cells when compared with $EpCAM^{+}$ tumor cells. The upregulated genes were clustered based on 10 different functions (apoptosis, drug resistance, stem cell markers, immune surveillance, metabolism, migration, cell signalling, proliferation, cell cycle, angiogenesis) related to the cancer progression. Subsequently, we validated the microarray data with three genes from each panel of four important functions. Taking into account the microarray data and qPCR validation, we propose that $EpCAM^{+}CD45^{+}$ cells have acquired gene expressions through gain-of-functions, further authenticated by functional assays. In view of the current findings, without ruling out the possibility of fusion with hematopoietic cells, we further propose that exosomes secreted by stromal cells might have role in influencing tumor cells and leading to the change in phenotype as well as subsequent functional gain. In this direction, we are pursuing investigation on the molecular cargo, secreted by tumour stromal cells. The exosomes from the ascetic fluid of human ovarian carcinoma have been isolated for further characterization.



# Chandrima Shaha

## 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. Regulatory networks driving cell fate decisions are important to investigate in the context of understanding diseases. The model systems used by us are a lower eukaryotic cell, the *Leishmania* parasite 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.

**Cell Death in protozoan parasites**

Investigations in parasite defense mechanisms have been a long-term goal of our work with the idea that insights into such functions would enable us to interfere with the function of vital enzymes required for survival of the parasite without interfering with the host system. The *Leishmania* parasites, not being naturally endowed with any canonical peroxidases such as catalase or selenium type glutathione peroxidase rely on the trypanothione dependent tryparedoxin peroxidase system for defense against peroxides. Continuing our studies on the tryparedoxin peroxidases, we sought to answer yet another unanswered question as to what are the relative functions of mTXNPx and cTXNPx in cell death in the face of oxidative stress produced at different sites. Two mutant parasites generated with compromised enzymatic activities of cTXNPx (cys52) and mTXNPx (cys81) were challenged with mitochondrially generated oxidative stress through inhibition of respiratory chain complexes and cytosolic oxidative stress. Parasites expressing competent mTXNPx

survived mitochondrial oxidative challenge better than those expressing mutant mTXNPx and were less capable of surviving cytosolic oxidative stress. The parasites expressing competent cTXNPx survived cytosolic oxidative challenge better than those expressing mutant cTXNPx and were less capable of surviving mitochondrial oxidative stress. This underscores the importance of local functions of these enzymes that are important because the parasites are exposed to severe cytosolic oxidative stress during infection. In another study, we show the involvement of ergosterol as a possible antioxidant molecule, a novel finding. On drug administration, ergosterol shows a prominent transient increase in the *Leishmania* parasite. This is important from the point of view of understanding the rampant drug resistance occurring with the various formulations and literature is very sparse on the subject. The importance of these studies lies in their utility to determine situations where parasites behave as sensitive or not sensitive to drugs or are infective or non-infective.

**Cell death in cancer**

Our models have been the embryonal carcinoma and acute myeloid leukemia cells used as *in vitro* cultures as well as tumors in xenograft models. We have shown that the partnering of p53 and Beclin-1 is an important determinant of cell fate in embryonal carcinoma cells. In the same cell type, the inactivation of mTORC1 dependent negative feedback loops is the main cause of cell death with rapamycin analogs in clinical trials. We used niclosamide, a specific inhibitor of mTORC1 that caused a significant reduction of tumor size. Since the role of p53 in mTORC2 inhibition was not known, we studied the effects and show that presence of p53 during inhibition of mTORC2 downregulates mTORC1 inhibition. In the absence of p53, inhibition of mTORC2 has no effect on mTORC1.

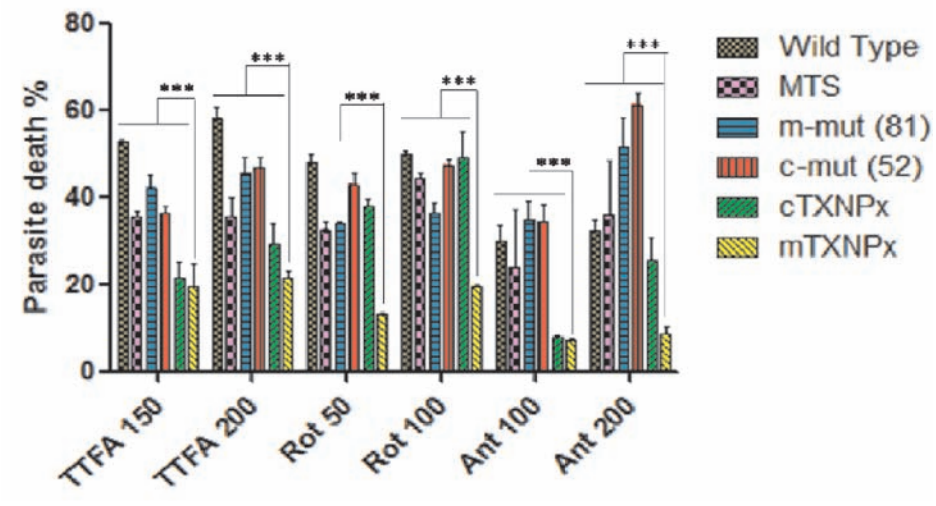



Figure 1. Figure showing cell death percentages in the various mutants and wild-type parasites in response to different mitochondrial respiratory chain inhibitors. MTS, parasites expressing only mitochondrial targeting signal (MTS), m-mut, mutation at cys-81, c-mut, mutation at cys-52, TTFA, 2-Thenoyltrifluoroacetone (Complex-II inhibitor), Rot, rotenone (complex-I inhibitor), Ant, Antimycin A (Complex-III inhibitor).

## Publications

### *Original peer-reviewed articles*


1. Ash D, Subramanian M, Surolia A, Shaha C (2015) Nitric oxide is the key mediator of death induced by fisetin in human acute monocytic leukemia cells. *Am J Cancer Res* **5**: 481-497.
2. Singh AK, Pandey RK, Siqueira-Neto JL, Kwon YJ, Freitas-Junior LH, Shaha C, Madhubala R (2015) A proteomic based approach to gain insight into reprogramming of THP-1 cells exposed to *Leishmania donovani* over an early temporal window. *Infect Immun* **38**:1853-1868
3. Bhattacharya K, Bag AK, Tripathi R, Samanta, SK, Pal BC, Shaha C, Mandal C (2014) Mahanine, a novel mitochondrial complex-III inhibitor induces G0/G1 arrest through redox alteration-mediated DNA damage response and regresses glioblastoma multiforme. *Am J Cancer Res* **4**: 629-647.
4. Tripathi R, Ash D, Shaha C (2014) Beclin-1 p53 interaction is crucial for cell fate determination in embryonal carcinoma cells. *J Cell Mol Med* **18:** 2275-2286.
5. Pandharkar T, Zhu X, Mathur M, Jiang J, Schmittgen T, Shaha C, Werbovetz KA (2014) Studies on the antileishmanial mechanism of action of the arylimidamide DB766: azole interactions and role of CYP5122A1. *Antimicrob Agents Chemother* **58**:4682-4689**.**


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1. Mathur R, Shaha C (2014) Cell death in a kinetoplastid parasite, the *Leishmania spp*. In *Leishmania*: Genomics, Molecular Biology and Control. (Eds: Adak, S and Dutta R) Horizon Scientific Press, United Kingdom. p 79-92.



# Satish Kumar Gupta

## Cellular and molecular aspects of reproduction and viral infection

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Development of contraceptive vaccine To enhance the immunogenicity and contraceptive efficacy of recombinant TT-KK-dZP3 [encompassing promiscuous T cell epitope of tetanus toxoid (TT; aa residues 830-844) followed by dilysine linker (KK) and ectodomain of dog zona pellucida glycoprotein-3 (dZP3; aa residues 23-348)], CpG motif was included along with alum. Inclusion of CpG resulted in an increase of serum IgG titers as well as contraceptive efficacy. Both systemic (intraperitoneal) and mucosal (intranasal) immune responses led to 50% decrease in fertility. In intraperitoneal group, serum/vaginal IgG titers of non-pregnant mice were significantly higher than pregnant mice. While in intranasal route, vaginal IgA seemed to be primary determinant for infertility (Fig. 1). These studies suggest that bioavailability of antibodies against TT-KK-dZP3 in female genital tract is important for contraceptive efficacy. In addition, a novel fusion protein encompassing fragment of dog ZP3 (aa residues 306-346), two copies of gonadotropin releasing hormone (GnRH) and 3 different promiscuous T cell epitopes was expressed in *E. coli*. The recombinant protein elicited antibodies reactive with ZP3 and GnRH in ELISA and 90% of the immunized female mice failed to conceive.

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

***i) Trophoblastic cell migration and invasion:*** During migration of HTR-8/SVneo trphoblastic cells, quantitative RT-PCR analysis revealed a significant increase in the expression of Wnt4 and Wnt11 in a scratch wound assay. Further, treatment of the cells with HGF led to an increase in the migration, which was also associated with increased expression of Wnt4 and Wnt11. These studies showed the importance of Wnt4 and Wnt11 during the migration of HTR-8/SVneo cells. Treatment of HTR-8/SVneo cells with LIF and EGF significantly increased their invasion whereas IFN-γ led to decrease in the invasion in Matrigel matrix invasion assay. Treatment with EGF leads to a significant increase in the phosphorylation of Akt, Erk1/2, STAT-1 and STAT-3. Experiments with Erk1/2 phosphorylation inhibitor, U0126, suggested the role of Erk1/2 phosphorylation in STAT-1 degradation and increased STAT-3 phosphorylation.

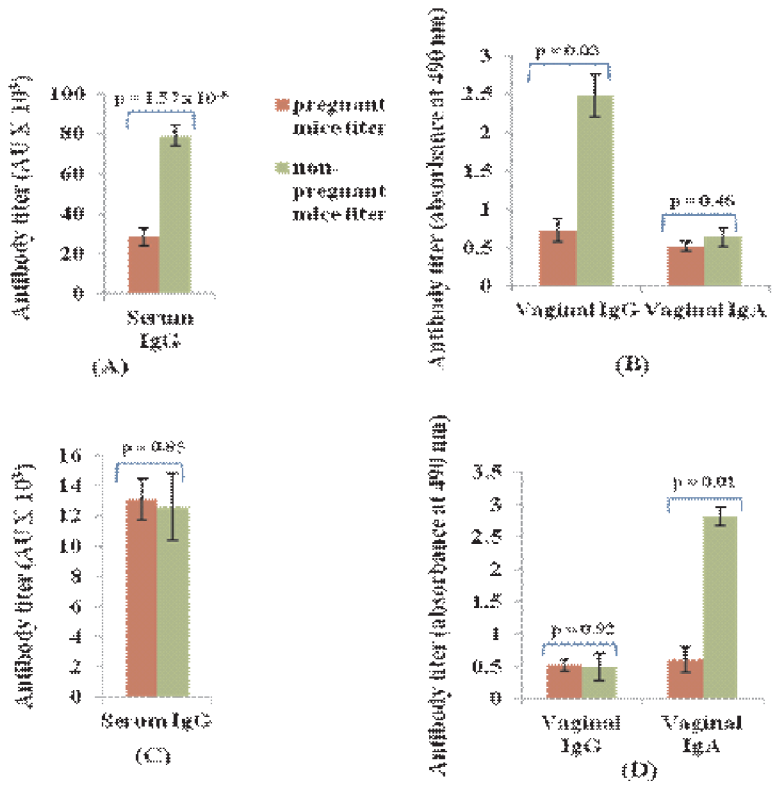



**Figure 1. Serum/vaginal IgG/IgA titer of pregnant/non-pregnant mice immunized with recombinant TT-KK-dZP3 supplemented with alum and CpG motif following two injections schedule:** Panels A & B show serum IgG and vaginal IgG and IgA titers for pregnant and non-pregnant mice immunized through intraperitoneal route with 50 µg of recombinant TT-KK-dZP3 supplemented with alum and CpG motif following two injection schedule. Panels C & D show serum IgG and vaginal IgG and IgA titers for pregnant and non-pregnant mice immunized through intranasal route in the same experiment.

***ii) Trophoblastic cell differentiation:*** We observed that forskolin mediated BeWo cell fusion is associated with an increased expression of active β-catenin and it may be associated with increased Wnt10b expression, independent of hCG regulation. We were able to rule out non-canonical activation and stabilization of β-catenin by phosphorylation at serine-675 via PKA in case of BeWo cell fusion by using H89 mediated PKA inhibition (Fig. 2).

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

The herbal formulation comprising of 50% ethanolic extracts prepared from stem bark of *Acacia catechu*, leaves of *Lagerstroemia speciosa* and *Terminalia chebula* showed dose dependent inhibition of HIV-1. It also showed potent inhibitory activity against HIV-1 integrase, reverse transcriptase and protease. Apart from the activity against HIV-1, the herbal formulation also exhibited anti-HSV-2 activity in plaque reduction assay using Vero cells. No significant reduction either in the viability of commonly found vaginal *Lactobacilli* strains or transepithelial resistance (TER) on the tight junctions formed by Caco-2 epithelial cells were observed in presence of the herbal formulation at 50 times the $IC_{50}$ concentration.

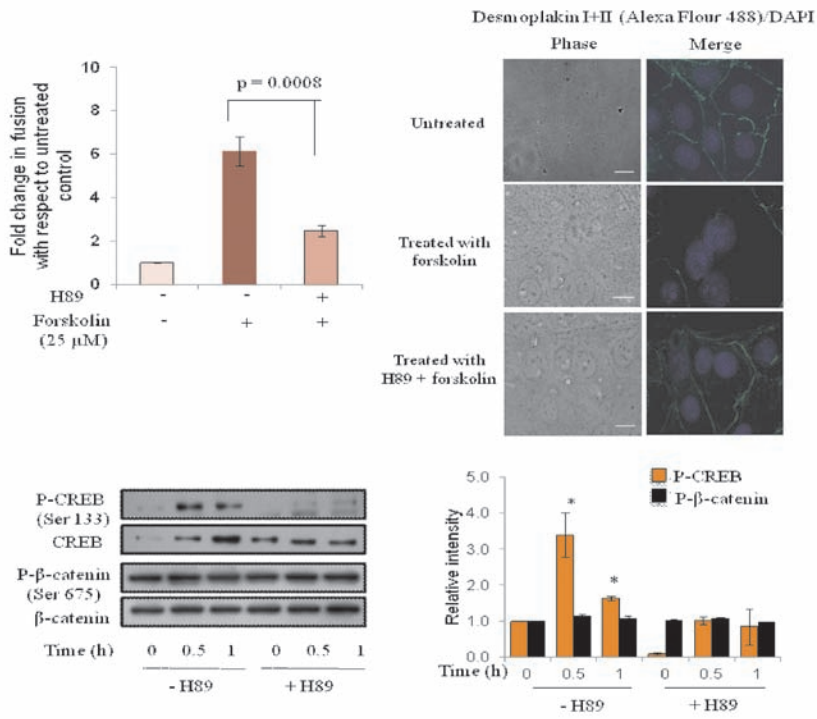



**Figure 2. Effect of PKA inhibitor on BeWo cell fusion:** BeWo cells were cultured in presence or absence of PKA inhibitor (H89; 10 µM) and cell fusion was assessed by Desmoplakin I+II staining. In addition, expression profile of P-CREB and P-β-catenin (Ser675) was assessed by Western blot.

## Publications

### *Original peer reviewed articles*

#1. Kushwaha RN, Debnath U, Singh P, Saxena R, Gupta SK, Tripathi RK, Siddiqui HH, Katti SB (2015) New piperazine-derived NNRTIs as anti-HIV agent: synthesis, biological evaluation and molecular docking studies. *Indo American Journal of Pharm Research* **5**:408-421.

2. Shrestha A, Srichandan S, Minhas V, Panda AK, Gupta SK (2015) Canine zona pellucida glycoprotein-3: up-scaled production, immunization strategy and its outcome on fertility. *Vaccine* **33**:133-140.

3. Shembekar N, Mallajosyula VVA, Piyush Chaudhury, Vaibhav Upadhyay, Varadarajan R, Gupta SK (2014) Design and characterization of a humanized monoclonal antibody that potently neutralize 2009 pandemic H1N1 virus. *Biotechnol J* **9**:1594-1603.

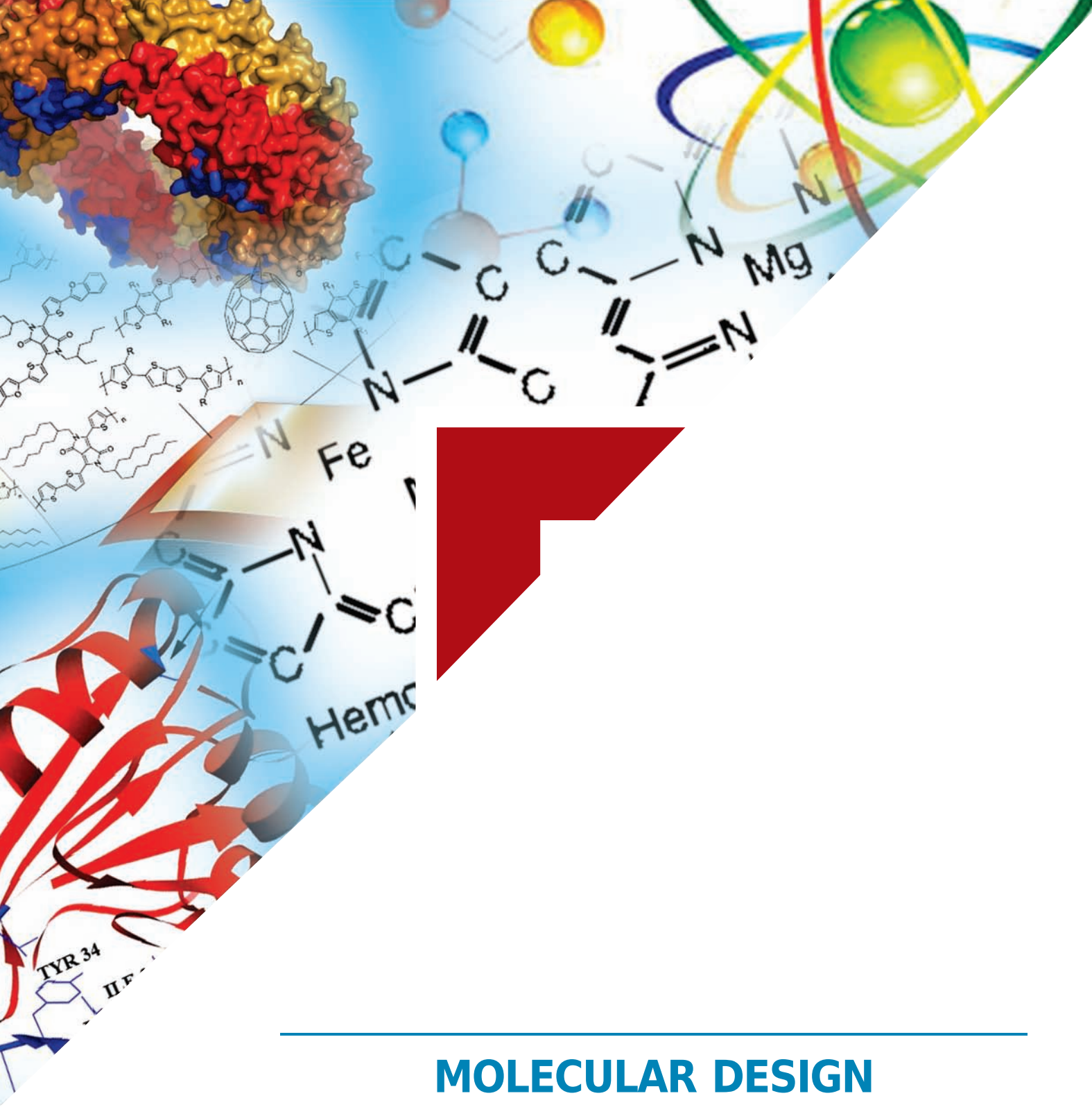
### *Reviews/Proceedings*

#1. Gupta SK (2015) Role of zona pellucida glycoproteins during fertilization in humans. *J Reprod Immunol* **108**:90-97.

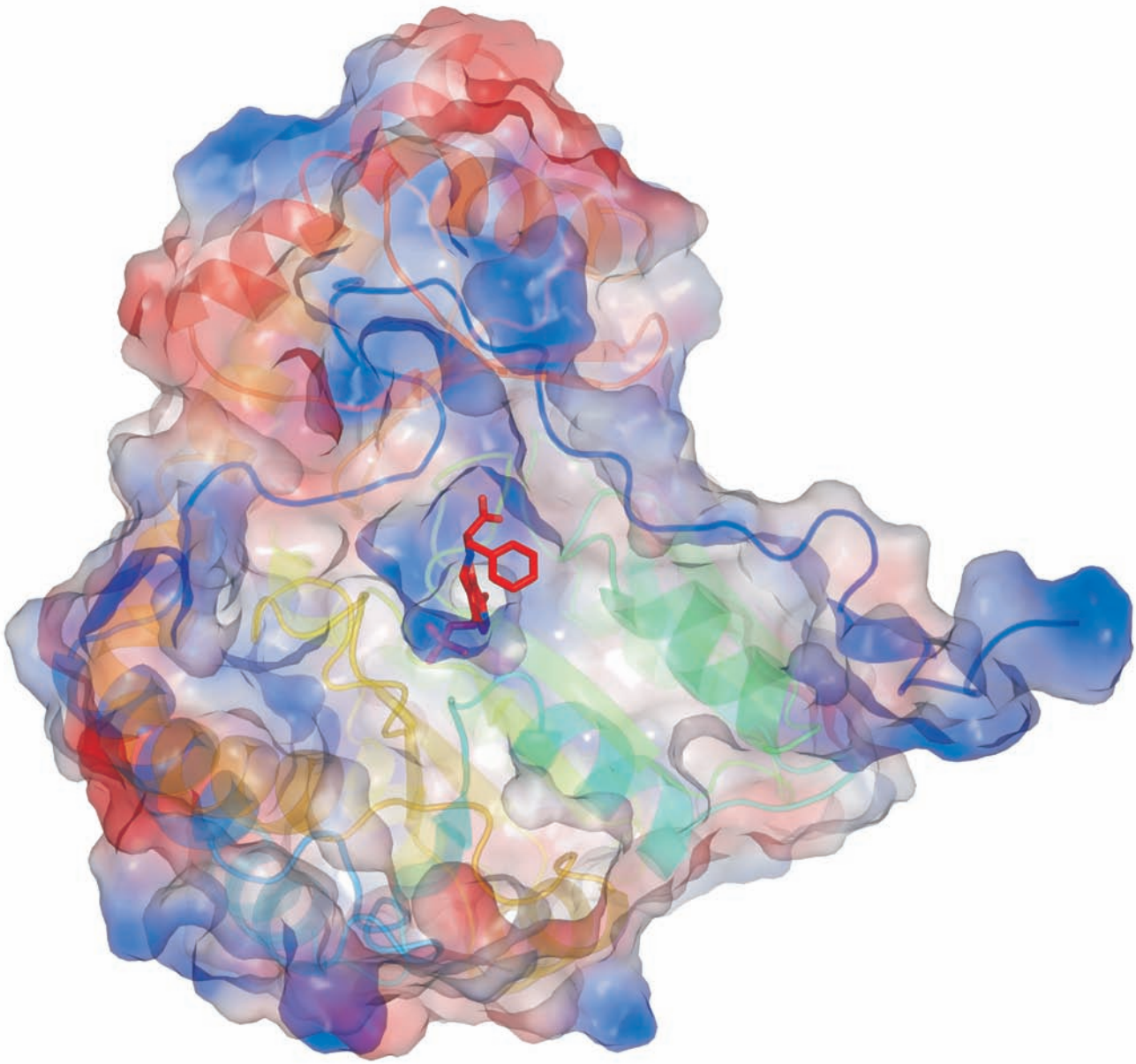
2. Gupta SK (2014) Unraveling the intricacies of mammalian fertilization. *Asian J Androl* **16**:801-802.

3. Gupta SK, Shembekar N (2015) Monoclonal antibodies. In, Textbook of Biochemistry and Human Biology (Talwar GP, Sarin SK, Hasnain SE eds). Prentice-Hall of India Private Limited, New Delhi, India. Chapter 106, p1233-1239.

#In Press on 31st March, 2014




# MOLECULAR DESIGN



Electrostatic potential surface of a monomeric ArAT with bound PLP and Phenylalanine in stick representation.

# MOLECULAR DESIGN


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# Monica Sundd

## Structural studies on proteins, dynamics and ligand interactions using NMR

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**Theme of research**

The theme of our research is to understand the structure, backbone dynamics and interactions of proteins using NMR, and other biophysical techniques to understand their function. We are working on some of the key proteins of fatty acid metabolism in *Leishmania viz*. the acyl carrier protein, acyl CoA binding proteins, and enzymes of type II fatty acid biosynthesis pathway.

**Objective**

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

Heterologous expression of *Leishmania* ACP in *E. coli* results in the expression of only one form of the protein, apo-ACP. Other type II ACPs express in two or more forms, apo-, holo- and acyl-intermediates. These results gave the first hint that the acyl carrier protein of *Leishmania* does not act as a good substrate for AcpS. With Sfp (a type II 4'-phosphopantetheinyl transferase, from *B. subtilis*), it shows several fold lower activity compared to *E. coli, P. falciparum and M. tuberculosis* ACP. Careful analysis of the NMR structure of the acyl carrier proteins suggested a remarkably different 4'-phosphopantetheinyl transferase (PPT) binding interface of *Leishmania* ACP. Type II ACPs have a conserved 'DSL' motif containing a serine. In *Leishmania* ACP, this DSL motif is replaced with an 'NSL' motif. Likewise, the conserved Met 44 is replaced with a Phe in *Leishmania* ACP and Glu 48 with a Gln.

***Leishmania ACP does not interact with E. coli AcpS.***

Wild type *Leishmania* ACP fails to convert into holo-ACP when expressed in *E. coli*. Mutagenesis studies were carried out in *Leishmania* ACP at the three positions (present in the PPT interaction interface), singly as well as in combination. The single mutants N36D, and F45M expressed in *E. coli* in a single form. However, the double mutant N36D+F45M

expressed in two forms, 78% apo and 22% holo-ACP suggesting that *E. coli* AcpS has a stringent requirement for the residues at the abovementioned positions.

***Enzymatic conversion of Leishmania ACP with B. subtilis Sfp***

We performed enzyme assays on wild type and the mutants using Sfp, a type II PPT from *B. subtilis*. As illustrated in Fig. 1, lowest $V_{max}$ was observed for the mutant F45M, displaying a $K_{cat}/K_M$ ratio (catalytic efficiency) 5 fold lower than wild type LmACP. A mutant F45A was also generated that failed to convert to holo-ACP suggesting that a hydrophobic anchor residue at position 45 in ACPs is necessary to interact with Sfp. The $K_{cat}$ values were in the order; F45M< Wild< N36D+Q48E>N36D+F45M< N36D< *P. falciparum* ACP< *E. coli* ACP< *M. tuberculosis* ACP as illustrated in figure. A mutant M44F of *E. coli* displays much higher $K_m$ of Sfp for CoA compared to wild type protein, but the $V_{max}$ remained unchanged.

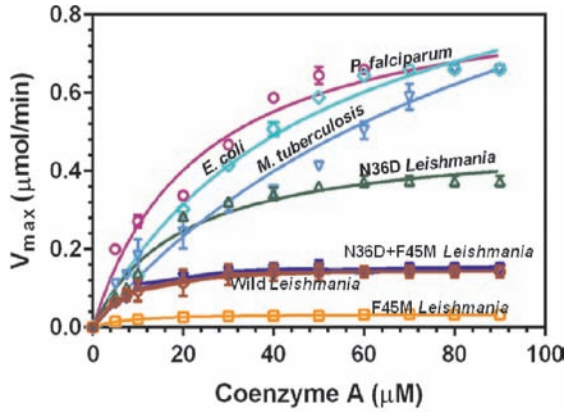
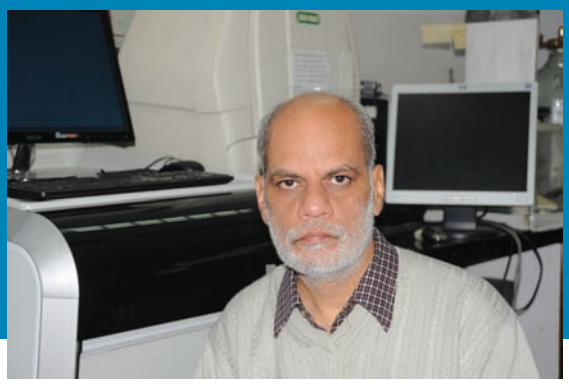



**Figure 1.** Enzyme kinetics curves for Sfp (*B. subtilis*) using various ACPs as substrates *viz. E. coli* ACP, *P. falciparum* ACP, *M. tuberculosis* ACP, *Leishmania* ACP and its mutant**s.**



# Pramod K. Upadhyay

## To develop strategies for making sensors and actuators for biological processes

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**Hepatocytes like cells from PBMCs**

To evaluate whether the *in vitro* generated hepatocyte like cells (NeoHeps) can be a good candidate for cell based therapy, NeoHeps were transplanted through spleen in a partially hepetectomised SCID mouse. Homing and engrafting of NeoHeps was determined in the regenerated liver tissue section by immunohistochemistry 10 days post transplantation.

The immunocytochemistry for human albumin and human connexin 32 in the tissue section of the NeoHeps transplanted hepatectomized SCID mouse liver confirmed the engraftment of human monocyte derived NeoHeps in the liver cortex (Fig. 1).

In order to confirm the functionality of the engrafted human NeoHeps in the mouse liver, the presence of human albumin was checked in mouse serum 10 days post transplantation by human albumin ELISA. The functional activity of transplanted NeoHeps, derived from monocytes isolated from peripheral blood, was evident from the presence of human albumin in the serum of the recipient SCID mouse detected by ELISA.

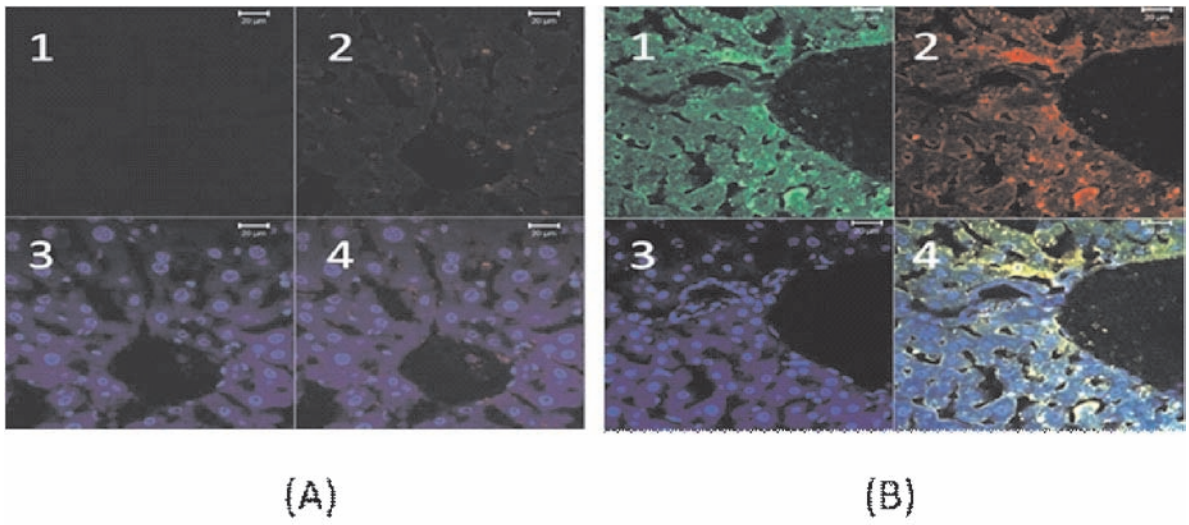



**Figure 1.** Confocal Image at 63X magnification of liver tissue section of (A) hepatectomized SCID mouse without cell transplantation and (B) hepatectomized SCID mouse injected with healthy NeoHeps. Connexin32 (Alexa Fluor 488), (2) Albumin (Alexa Fluor 594), (3) Nucleus (DAPI) and (4) merge picture.

To confirm the engrafted cells in the mouse liver tissue section were of human origin, fluorescence in situ hybridization (FISH) was performed with DNA probe specific to human centromeric region of chromosome 9. Binding of DNA probe specific to human chromosome 9 centromeric regions in the nucleus of the liver tissue section of NeoHep transplanted hepatectomized SCID mouse further confirmed the engraftment of human origin cell in the mouse liver cortex (Fig. 2).

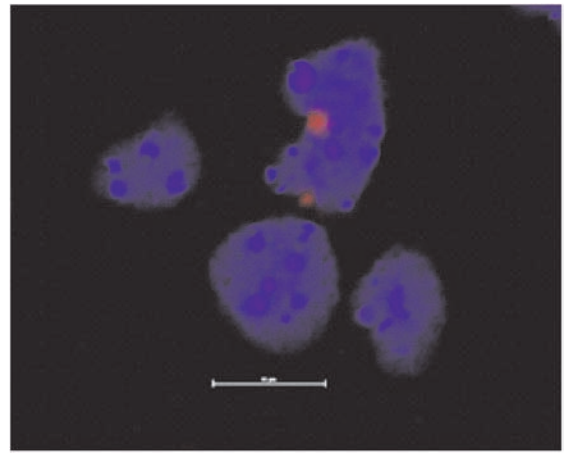


**Figure 2.** Fluorescence In Situ Hybridization on NeoHep transplanted 1/3rd partial hepatectomized SCID liver section by probe specific to human chromosome 9 centromere. The probe was biotinylated and streptavidin AlexaFluor 594 was used for detection.

**Differentiation of PBMCs into retinal neuron like cells**

The retina is one of the most complex high cell density tissues with high metabolic activity and has a perfect integrity despite all its randomness. We utilized the two steps procedure, in the first step; the monocytes were de-differentiated into stem cell like cells called reprogrammed monocytes (RM). In the next step, the reprogrammed monocytes were differentiated into retinal neuron like cells by culturing them in medium containing specific growth factors to obtain retinal neuron like cells (RNLC).

We have developed an immuno-compromised mouse model for retinitis pigmentosa, NOD

scid-CBA/J pde6b rd1 to test and validate the function of RNLC derived from human PBMCs.

## Publications

### *Original peer-reviewed articles*


1. Iyer S, Arindkar S, Mishra A, Manglani K, Kumar JM, Majumdar SS, Upadhyay P*, Nagarajan P* (2015) Development and Evaluation of Transgenic Nude Mice Expressing Ubiquitous Green Fluorescent Protein. *Mol Imaging Biol* **17**: 471-478.
2. Tyagi P, Gupta N, Jain A, Upadhyay P, Puliyel J (2015) Intra-gastric Pressures in Neonates Receiving Bubble CPAP. *Indian J Pediatr* **82**:131-135.


### *Review/Proceedings*


1. Jain Y, Upadhyay P (2014) An inexpensive and 'do it yourself' infant warmer without electricity. *Natl Med J India*. **27**:55.


### Patent


1. Bhattacharjee J, Das B, Nagarajan P, Upadhyay P (2015) A simple device for injecting small volume (5-100µl) of liquid in small laboratory animals. (415/DEL/2015, Indian patent filled on February 13, 2015).


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# Rajendra P Roy

## Protease-catalyzed splicing of peptide bond

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We study the principles underlying peptide ligation reactions catalyzed by proteases and transpeptidases with a view to apply them to the semi-synthesis of proteins and assembly of well-defined bioconjugates. Transpeptidase sortase that catalyzes covalent anchoring of surface proteins to the cell wall in gram-positive bacteria has turned out to be a wonderful enzyme in this endeavor.

**Structure, dynamics and function of sortases**

Sortases are classified as A to F based on their membrane topology and substrate specificity. The ability of prototypical sortase A (SrtA) of *Staphylococcus aureus* to ligate LPXTG nested proteins to an aminoglycine derivatized moiety has enabled myriad biotechnological applications. However, newer challenging applications would require availability of sortases with orthogonal specificities. In this connection, it is pertinent to note that some bacteria encode class E (SrtE) and F (SrtF) sortases, which are annotated to process non-canonical or *hitherto* unknown peptide substrates.

The *S. avermitilis* genome predicts the presence of at least four Class E sortases. One of the predicted class E sortase was cloned, expressed and purified. Preliminary biochemical characterization revealed that SrtE efficiently transferred noncanonical LPXTG peptide substrates to non-Gly nucleophiles. The enzyme poorly accepted LPXTG substrates.

*Therrmobifida fusca* genome encodes for a single sortase, which is classified as SrtF. For ease of expression and purification, a truncated version of this sortase was expressed with a N-terminal His tag. Preliminary transpeptidation assays revealed that SrtF transferred canonical as well non-canonical LPXTG substrates to Gly-nucleophiles.

Contemporaneous with this, structural studies on SrtA of *Streptococcus pneumoniae* was elaborated to glean further insights in to the action mechanism of this enzyme. The *pneumoniae* SrtA exists as a dimer in solution and forms intertwined domain swapped dimers in crystals. Interestingly, active site residues (H141, C207 and R215) lie on an open interface in 3D structure. Curiously, R215A mutant predominantly formed monomers in solution. Mutational studies of interacting residues of R215 located in the β7/β8 loop were carried out to disentangle its role in catalysis *vis a vis* maintenance of quaternary structure.

**Sortase-mediated protein labeling and conjugation**

We have also been seeking strategies for semisynthesis of Ubiquitin (Ub) or SUMO conjugates that can serve as substrate for deubiquitinating or desumoylating enzymes. The generation of Ub/SUMO conjugates in useful amounts for *in vitro* studies is complicated by the involvement of multi-steps and the occurrence of myriad variants of associated ligases. Inspired by the fact that sortase-catalyzed transpeptidation entails peptide ligation to a Gly-Gly motif, we initiated studies to explore if sortase can be useful in the semisynthesis of Ub/SUMO conjugates. Toward this, commercial Ub and SUMO clones were suitably modified to express a hexa-His tagged sortase-recognizable protein. For ligating a bona fide peptide substrate to Ub and SUMO proteins, we considered peptides derived from p53 as target for ubiquitylation and sumoylation. The peptides carrying isopeptide linked di-Gly residues to the target lysine residue in p53 were assembled exploiting an orthogonal protecting group. Peptide conjugation to Ub or SUMO is in progress.

**Publication**

***Original peer-reviewed article***

1. Biswas T, Pawale VS, Choudhury D, Roy RP (2014) Sorting of LPXTG peptides by archetypal Sortase A: Role of invariant substrate residues in modulating the enzyme dynamics and conformational signature of a productive substrate. *Biochemistry* **53**: 2515-2524.



# Sarika Gupta

## Therapeutic Interventions in Chronic Diseases

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To design and synthesize stable curcumin analogs and study their effect on aggregation and cytotoxicity of Wild type and Mutant α-Synuclein.

Accumulating evidence suggests that deposition of neurotoxic α-synuclein aggregates in the brain during the development of neurodegenerative diseases like Parkinson's disease can be curbed by anti-aggregation strategies that either disrupt or eliminate toxic aggregates. Curcumin, a dietary polyphenol exhibits anti-amyloid activity but the use of this polyphenol is limited owing to its instability. As chemical modifications in curcumin confiscate this limitation, such efforts are intensively performed to discover molecules with similar but enhanced stability and superior properties. This study focuses on the inhibitory effect of two stable analogs of curcumin viz. curcumin pyrazole and curcumin isoxazole and their derivatives against α-synuclein aggregation, fibrillization and toxicity. Employing biochemical, biophysical and cell based assays we discovered that curcumin pyrazole (3) and its derivative N-(3-Nitrophenylpyrazole) curcumin (15) exhibit remarkable potency in not only arresting fibrillization and disrupting preformed fibrils but also preventing formation of A11 conformation in the protein that imparts toxic effects. Compounds 3 and 15 also decreased neurotoxicity associated with fast aggregating A53T mutant form of α-synuclein (Fig). These two analogues of curcumin described here may therefore be useful therapeutic inhibitors for the treatment of α-synuclein amyloidosis and toxicity in Parkinson's disease and other synucleinopathies.

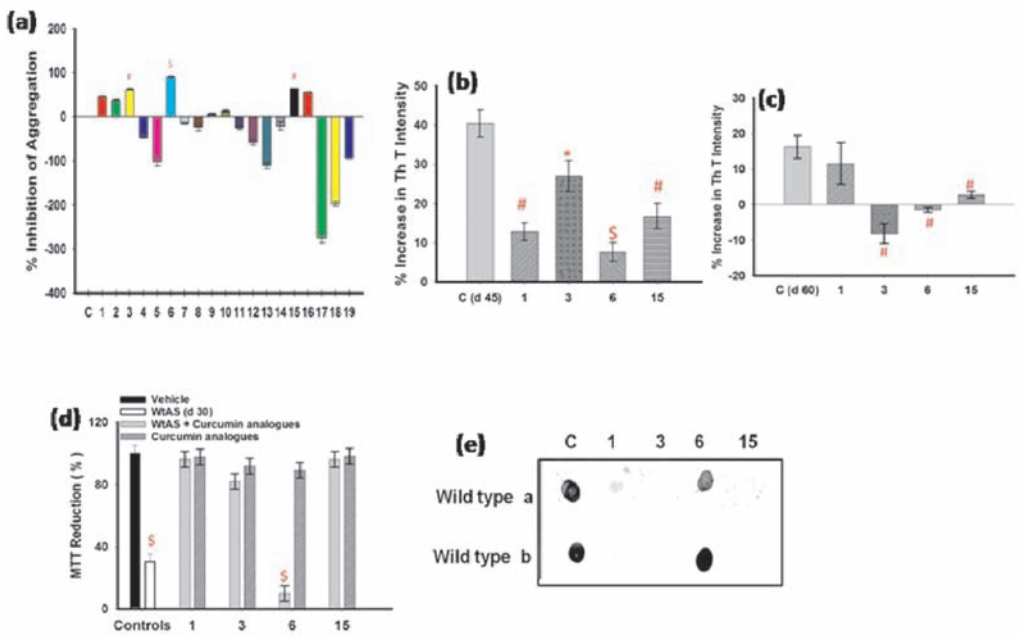



Figure 1. (a) Percentage inhibition of aggregation of WtAS by compound 1-19 as monitored by ThT assay. (b) Comparative bar diagram showing the effect of compounds 1, 3, 6 and 15 on 15 days (c) and 30 days aggregated WtAS. (d) Representative image of A11 dot blot showing effect of compounds 1, 3, 6 and 15 on WtAS A11 epitope formation. (a) WtAS (210 µM) aggregated alone or co-incubated with equimolar concentration of compound 1, 3, 6 and 15 for 30 days (b) 60 days. The compounds were added at day 15 or 30 and further co-incubated for 30 days.(e) Comparative bar diagram showing MTT reduction by oligomers of WtAS(210 µM) alone or co-incubated with compounds 1, 3, 6 and 15 in neuronal cell cultures. Results are the mean of three different experiments done in duplicates and error bars show standard deviation. *, #, $ denote $p < 0.05$, $p < .005$ and $p < 0.0001$, respectively.

## Publication

### *Original peer-reviewed article*


1. Pasi S, Kant R, Gupta S, Surolia A (2015) Novel multimeric IL-1 receptor antagonist for the treatment of rheumatoid arthritis. *Biomaterials* **42**:121-133.


## Patents


1. Surolia A, Gupta S, Singh MP, Chattopadhyay T (2014). Composition useful for treatment of diabetes & disorder. Australian patent No. 2009200906.
2. Surolia A, Gupta S, Singh MP, Chattopadhyay T (2014). Composition useful for treatment of diabetes & disorder. Australian patent No. 2008354530.
3. Surolia A, Gupta S, Singh MP, Chattopadhyay T (2014). Process for preparing supramolecular calcitonin assemblies (SCA). Europian Patent no EP2282763 (validated in UK and Germany).



# Apurba Kumar Sau

## Molecular mechanism of enzymatic reactions and enzyme-ligand interactions

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**Circular dichroism study to understand the role of the insertion motif in *H. pylori* arginase**

To examine the role of the insertion motif in secondary structure, CD measurements were performed. Trp159Ala and Glu155Ala showed significant decrease in the CD value compared to wild type, suggesting that these mutations lead to the loss of secondary structure. The deconvolution yielded an average α-helix content of ~ 6.3 and 18% for Trp159Ala and Glu155Ala, respectively as compared to 29% in wild type. To understand whether Trp159 has a role in the structure, similar studies were performed with Trp159Phe. Unlike Trp159Ala, Trp159Phe regained secondary structure. These data demonstrate that both Trp159 and Glu155 are individually important for maintaining the structure. But other mutants exhibit similar results to wild type.

To understand whether the loss of activity in Glu155Ala is due to the decrease in the helical content, CD measurement of Glu155Ala was performed with 5-10 % TFE. The secondary structure with 10% TFE was similar to wild type. The activity assay of Glu155Ala with 10% TFE showed negligible activity (~ 2% of wild type), suggesting that Glu155 has a catalytic role.

**Heat-induced denaturation studies for the role of the sequence motif**

To determine whether the motif is important for stability, heat-induced unfolding studies were performed. Comparison of the $T_m$ values between the wild type and mutants showed that in the absence of the metal ions the mutant proteins are significantly less stable than the wild type (reduction in the $T_m$ by ~ 5-17$^0$). Interestingly, only Glu155Ala and Trp159Ala showed the notable decrease in the $T_m$ (~14-17°) compared to wild type, suggesting that both electrostatic (by Glu155) and hydrophobic (by Trp159) interactions are very critical in providing stability to the apo-proteins. To see

the involvement of Trp159 in these interactions, the stability of Trp159Phe was examined. An increase in the $T_m$ of about 9 degree was observed compared to Trp159Ala, suggesting the role of Trp159 in hydrophobic interactions for the stability. Unlike other mutants, the metal ions in Glu155Ala did not compensate the loss of stability significantly compared to their apos, suggesting that the interaction of Glu155 with its surrounding residues is vital for stability. Thus, this study clearly shows that the motif is a critical component for the stability of *H. pylori* arginase.

**Higher GMP formation is regulated through tetramerization of hGBP1**

To understand whether the enhanced GMP formation in hGBP1 is regulated through a higher order assembly, analytical gel-filtration assay was performed with the wild type, mutant and truncated proteins in the absence and presence of the transition state analogue, GDP.$AlF_4$. With GDP.$AlF_4$, hGBP1 eluted as a tetramer. We performed similar assays with a series of the mutants, D108A, D103L.D108L, T75A and R48P, where these residues are known to be primarily critical for GMP formation. Interestingly, with GDP.$AlF_4$ D108A, which forms significantly reduced GMP compared with wild type, eluted as a mixture of monomer and tetramer. In contrast, D103L.D108L, which was completely impaired in GMP formation, eluted only as a monomer, indicating that the double mutant did not tetramerize with GDP.$AlF_4$. R48P is known to have a negligible GTP binding or hydrolysis, whereas T75A hydrolyzes GTP to mainly GDP. With GDP.$AlF_4$, R48P and T75A eluted as a monomer and dimer, respectively, indicating that none of the two mutants tetramerize with GDP.$AlF_4$. Importantly, hGBP1$^{307}$, which is known to produce lower GMP, eluted only as a dimer with GDP.$AlF_4$, suggesting an important role of the helical domain in tetramer formation. Taken together, these data suggest that the enhanced GMP formation is associated with tetramerization of the protein.



# Janendra K. Batra

## 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. Human ribonucleases and naturally occurring protein toxins, are being explored to design knowledge-based recombinant toxins.

2. Investigation of crucial housekeeping proteins of *M. tuberculosis* for their role in survival and virulence of the pathogen. Caseinolytic protease (Clp) regulates the expression of virulence genes, and also helps bacterial pathogens in countering stress in the host. RNase P is structurally completely different in bacteria and human. The functioning of Clp machinery and RNase P mediated tRNA maturation is being investigated in *M. tuberculosis.*

***Stress regulation and persistence mechanisms in Mycobacteria***

We have further characterized the *M. tuberculosis* transcriptional repressor, HspR (MtHspR) for its biochemical properties and also investigated the mechanism by which it may function under heat stress. We have demonstrated that HspR of *M. tuberculosis* independently binds to the HAIR DNA element. The HAIR binding activity of HspR was intact upto 60°C indicating that MtHspR on its own is not the sensor of heat stress. We found proteins from bacterial cell extract to make the MtHspR-DNA interaction heat responsive. We demonstrate DnaK to bind the *M. tuberculosis* HspR-HAIR complex and the monomeric form of DnaK is most favored state. The study also

indicates that *M. tuberculosis* DnaK-HspR-HAIR binding is affected by heat stress, especially so in the presence of the substrate protein, α-casein, which supports the feedback loop mechanism.

***Structure-function analysis of Clp proteins of M. tuberculosis***

To understand the mechanism of ClpB chaperone mediated protein disassembly in *M. tuberculosis*, we cloned and expressed, in *E. coli,* the proteins of *M. tb* ClpB and DnaKJE chaperone complex namely, ClpB, ClpBΔN, DnaK, DnaJ1 and GrpE. Both, ClpB and ClpBΔN showed a mixed population of hexameric and monomeric proteins and showed inherent ATPase activity. The ATPase activity of ClpB and ClpBΔN were differently modulated in the presence of protein substrates. ClpB showed a high prevention of aggregation activity compared to that of ClpBΔN.

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

In an attempt to reconstitute the *M. tuberculosis* RNase P *in vitro*, we expressed the protein subunit in *E. coli* and purified it from the inclusion bodies. The purification was achieved using two different strategies. The two proteins differed remarkably in their properties. The protein that was purified after renaturation showed a significant and specific inherent RNase P activity, whereas, the protein purified under denaturing conditions and renatured subsequently did not contain any inherent RNase P activity. It appears that the *M. tuberculosis* RNase P protein subunit is capable of attaining a conformation *in vitro* that is catalytically competent. Further validation of these observations is underway.

The various mutants of RNase Protein, generated earlier were purified under denaturing conditions and further characterized for catalysis. The study shows that in *M. tuberculosis* RNase P protein subunit residues V27, A70 and R72 are involved in substrate recognition; H67, F23, A77 and R93 are involved in providing the RNA subunit an active conformation in the holozyme. Attempts are on to generate a conditional RNase P protein knockout strain of *M. tuberculsis* to investigate the role of protein subunit in RNase P catalysis *in vivo*.

**Publications**

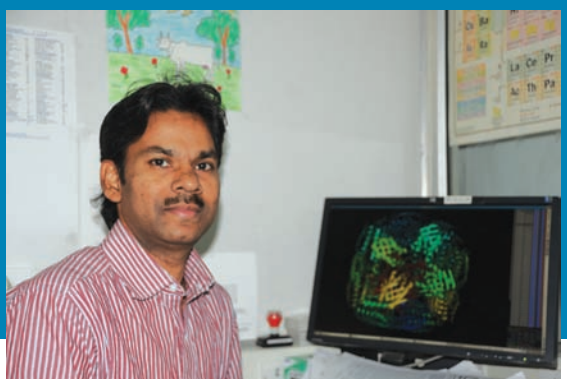
***Original peer-reviewed articles***


1. Yadav SK, Batra JK (2015) Ribotoxin restrictocin manifests anti-HIV-1 activity through its specific ribonuclease activity. *Int J Biol Macromol* **76**:58-62.
2. Haque MA, Shah U, Zaidi S, Hassan MI, Islam A, Batra JK, Ahmad F (2015) Characterization of pre-molten globule state of yeast iso-1-cytochrome *c* and its deletants at pH 6.0 and 25°C. *Int J Biol Macromol* **72**: 1406-1418.
3. Haque MA, Zaidi S, Shah U, Prakash A, Hassan MI, Islam A, Batra JK, Ahmad F (2014) *In vitro* and *in silico* studies of urea-induced denaturation of yeast iso-1-cytochrome *c* and its deletants at pH 6.0 and 25°C. *J Biomol Struct Dyn* **33**: 1493-1502.
4. Ubaid-Ulah S, Haquea MA, Zaidi S, Hassan MI, Islam A, Batra JK, Singh TP, Ahmad F (2014) Effect of sequential deletion of extra N-terminal residues on the structure and stability of yeast iso-1-cytochrome-c. *J Biomol Struct Dyn* **32**: 2005-2016.


***Review/Proceedings***


#1. Chopra A, Batra JK (2014) Antimicrobial activity of human eosinophil granule proteins. *Methods Mol Biol* **1178:** 267-281.


# In press last year since published.



# Bichitra K. Biswal

## Understanding structures and functions of *Mycobacterial* protiens

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Deepak Kumar

We have been pursuing two research projects to decipher structural and functional aspects of proteins from *Mycobacterium tuberculosis (Mtb)*, the organism that causes tuberculosis (TB) in humans. The first aims at elucidating the 3D structures and biochemical properties of enzymes of histidine (His) biosynthesis pathway to unravel the molecular mechanisms underlying their actions and to design enzyme specific anti-TB small molecule compounds through a structure-based approach. A similar line of study on membrane-associated proteases forms the basis of the second project.

The histidine biosynthesis pathway, the synthesis of histidine from 5-phosphoribosyl-1-pyrophosphate in 10 enzymatic steps by 10 enzymes, is conserved among bacteria, lower eukaryotes and plants, but is absent from higher eukaryotes including mammals. We have been studying structural and biochemical aspects of these enzymes from *M. tuberculosis,* the organism that causes tuberculosis (TB) in the context of deciphering the molecular mechanisms underlying their actions and designing enzyme-specific anti-TB inhibitors. Previously we have determined 3D structures of HisB (imidazole glycerol phosphate dehydratase) and HisC (a histidinol phosphate aminotransferase) and have characterized these enzymes biochemically. We also have shown that a triazole scaffold compound, 3-amino-triazole, is a competitive inhibitor of HisB. In the past one year, we have determined 3D structures of apo and ligand-bound forms of another enzyme, a PLP-dependent aromatic amino acids aminotransferase (ArAT). The functional unit of this enzyme is a dimer (Fig. 1) and each monomer consists of three domains, palm, thumb and fingers. The PLP-binding domain in the palm position (palm domain) encompasses a major portion of the polypeptide chain and consists of a seven-stranded β-sheet sandwiched between two bundles, each with three α- helices. The C-terminal domain resembling the fingers (fingers domain) forms a roof-shaped three-helix

bundle resting over a small β-sheet. An N-terminal 'lid', consisting of an approximately 40-residue long loop, protrudes from the thumb domain and closes over the PLP-binding domain. Of note, structural analyses of ArAT-Phe bound complex reveals that the hydrophobic nature of the substrate binding pocket (Fig. 2) and the N-terminal lid of ArAT facilitates the binding of Phe and other aromatic residues such as Tyr and Trp. We are currently exploiting these data to design enzyme specific inhibitors. X-ray data from crystals of a truncated version of a membrane protein (Rv2224) were collected and the structure is being solved.

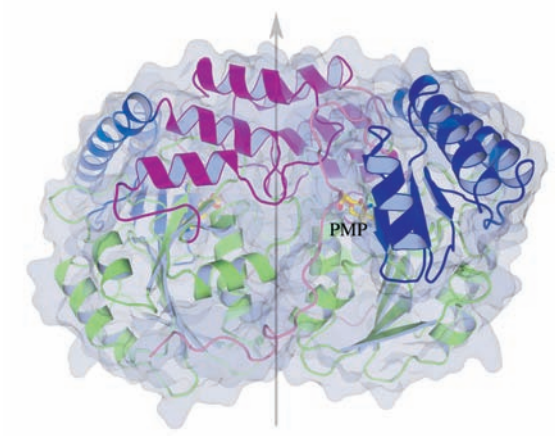



**Figure 1 -** The 3D structure of ArAT shown in ribbon and surface representations. The vertical arrow shows the 2-fold rotation axis that relates one monomer with the other. Also shown in the figure is a bound PMP molecule, in the active site of ArAT, in stick representation.

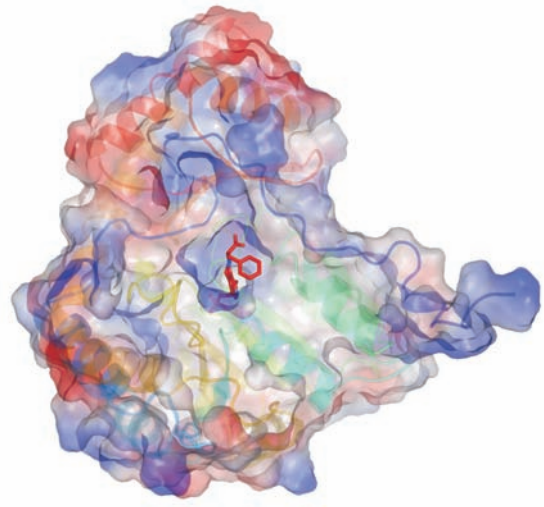


**Figure 2 -** Electrostatic potential surface of a monomeric ArAT with bound PLP and Phenylalanine in stick representation.

## Publication

### *Original peer-reviewed article*


**1.** Saroj DC, Singh KH, Anant A, Biswal BK (2014) Overexpression, purification, crystallization and structure determination of AspB, a putative aspartate aminotransferase from *Mycobacterium tuberculosis*. *Acta Crystallogr F Struct Biol Commun* **70:**928-932.



# Debasisa Mohanty

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

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The main theme of the research projects is to understand the structural principles that govern folding of peptides/proteins to stable conformations and binding of various ligands to proteins, and use these principles for developing novel computational approaches for prediction of the structures of peptides/proteins and specificities of protein-ligand complexes. The specific objective of the various projects are to investigate, whether the combination of knowledge-based and *ab initio* approaches can be used for predicting the (1) substrate specificity of enzymes involved in novel post-translational modifications (2) substrate specificity of various peptide recognition modules (PRMs) like MHCs, kinases, PTB, PDZ and WW domains etc.

**A. *Analysis of enzymes associated with novel post-translational modifications***

Fic domain was recently shown to catalyze AMPylation - transfer of AMP from ATP to hydroxyl side chain of diverse eukaryotic proteins, ranging from RhoGTPases to chaperon BiP. We have carried out a series of explicit solvent molecular dynamics (MD) simulations upto 1 μs duration on apo, holo and substrate/product bound IbpA Fic domain (IbpAFic2). Comparative analysis of the MD trajectories revealed how binding of ATP allosterically induces conformational changes in the distal switch II binding region of Fic domains thereby aiding in substrate recognition. Our simulations have also identified crucial aromatic-aromatic

interactions, which stabilize the orientation of the catalytic histidine for inline nucleophilic attack during AMPylation, thus providing structural basis for evolutionary conservation of these aromatic residue pairs in Fic domains (Fig. 1). Based on analysis of interacting interface residue pairs, which persist over the microsecond trajectory, we identified a tetrapeptide stretch involved in substrate recognition. In additions, combined use of simulations and phylogenetic analysis has helped in discovery of a new subfamily of Fic proteins, which harbor a conserved Lys/Arg in place of inhibitory Glu of the regulatory helix.

**B. Analysis of internal peptide recognition by PDZ domains**

Deciphering interaction partners for PDZ domains is a topic of considerable current interest, because of involvement of these modular recognition domains in many human diseases. PDZ domains interact with the short peptides present at the C-terminus of interaction partners. However, internal peptides can also be recognized by many PDZ domains. Earlier we have developed structure-based methods for predicting interaction partners of PDZ domains, which recognize C-terminal peptides. Crystallographic studies

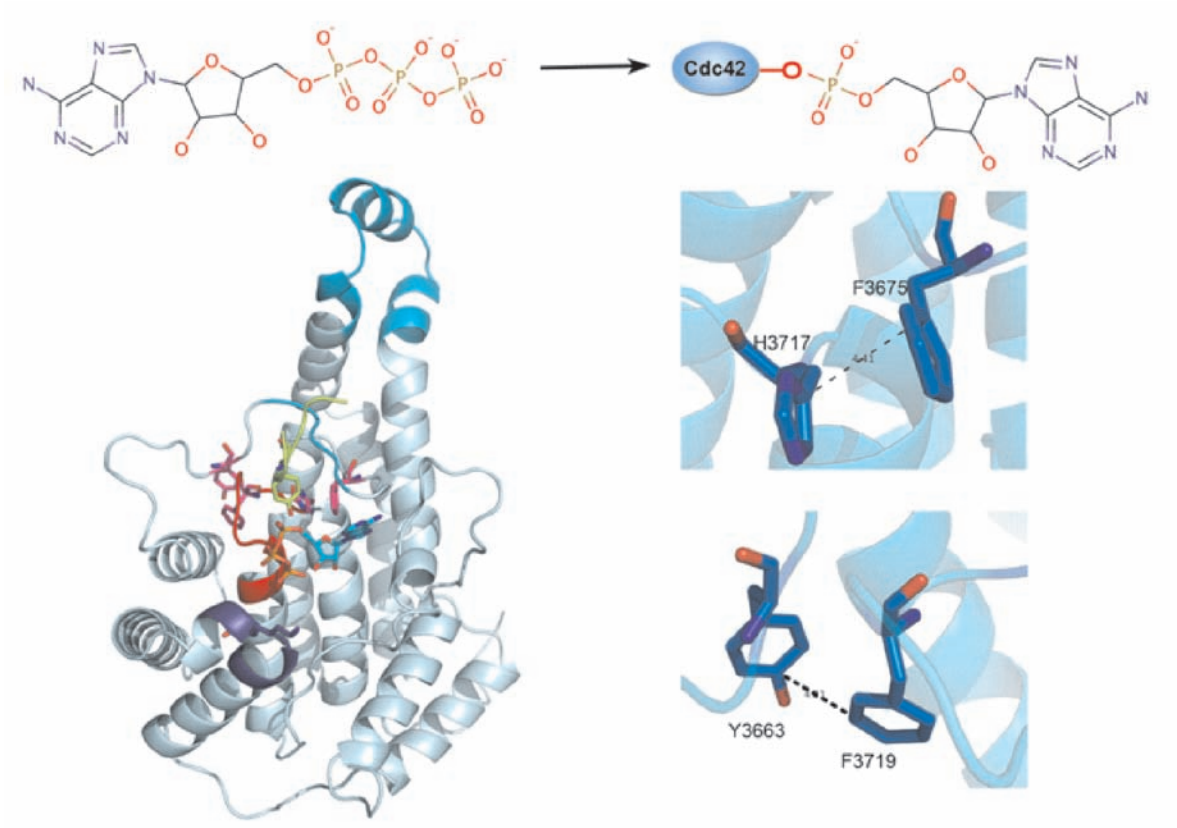



**Figure 1.** MD simulations reveal role of aromatic interactions in AMPylation reaction

have revealed that, certain PDZ domains utilize two different conformations to interact with C-terminal or internal peptides. We have attempted to develop computational methods for predicting non-canonical binding partners of PDZ domains by analyzing the mechanistic details of internal peptide recognition which is facilitated by the plasticity of the PDZ domain. A series of 1μs explicit solvent MD simulations have been performed on cognate and non-cognate complexes of the Par6 PDZ domain. Analysis of these MD trajectories revealed that the non-cognate complexes generated by interchanging the bound peptides in the crystal structures converged to the conformations in cognate complexes during the 1 μs simulation. Thus, our MD simulations were able to reproduce the conformational transitions involving movement of the carboxylate binding loop, which is required for recognizing internal peptides. It was found that in absence of bound peptide carboxylate binding loop of Par6 PDZ domain adopts a closed conformation and internal peptide recognition follows induced-fit mechanism.

**Publications**

***Original peer-reviewed articles***


1. Khater S, Mohanty D* (2014) Genome-Wide Search for Eliminylating Domains Reveals Novel Function for BLES03-Like Proteins. *Genome Biol Evol* **6**:2017-2033.
2. Damle NP, Mohanty D* (2014) Mechanism of autophosphorylation of mycobacterial PknB Explored by molecular dynamics simulations. *Biochemistry* **53**:4715-4726.
3. Kumar N, Damle NP, Mohanty D* (2015) Getting phosphorylated: Is it necessary to be solvent accessible? *Proc Indian Natn Sci Acad* **81**:493-507.



*Corresponding author.



# Dinakar M. Salunke

## Structure, interaction and design studies involving regulatory peptides and proteins

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**Project Associate**
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**Project Assistant**
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**Collaborator**
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**Theme of research**

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

**Objectives**

1. Understanding the protein architecture and the structural biology of various regulatory events.
2. Analysis of the structural principles of immune recognition and molecular mimicry.
3. Rational molecular design studies based on the above.

Extensive studies have been done in this laboratory to understand the mechanism(s) underlying the recognition of chemically dissimilar ligands by a common immune receptor clearly proves that breakdown in the antigenic discrimination potential and therefore degeneracy is often encountered in the immune system. These degenerate antibodies might have an edge over a fast mutating virus such as Influenza A virus.

To address this issue we started screening the Tomlinson I + J human single fold synthetic naïve phage display single chain antibody fragment libraries against a highly mutated continuous epitope of Influenza A virus i.e. WTGVTQN. In this library, DNA encoding millions of variable heavy ($V_H$) and variable light ($V_L$) chains linked by flexible glycine-serine linker products are cloned into a vector which is then engineered to express scFv fused to pIII minor capsid protein of filamentous bacteriophage of *E. coli*. The peptide was synthesized and conjugated to the proteins KLH and BSA by glutaraldehyde conjugation

method. Phages carrying target antibody were isolated by four cycles of selection against KWTGVTQN-BSA and KWTGVTQN-KLH conjugates. Eluted phages were allowed to infect TG1 bacteria in order to isolate individual phagemid clones. These individual clones were screened for their ability to bind the peptide epitope by monoclonal phage ELISA and competitive ELISA. Positive clones were further confirmed by PCR to check for the presence of full length $V_H$ and $V_K$. Degenerate binding specificities were analyzed against 8 different analogues of the peptide. Currently, few selected clones are being subjected to expression in soluble scFv form.

Observed cross-reactivity as seen in our work on both germline and affinity-matured antibodies is in defiance to the conventional rules of specificity. Crystallographic studies on antibody diversity have given newer insights into the mechanism of repertoire amplification at the germline as well as at the matured stage offering interesting physiological perspectives bringing out intriguingly new aspects of antigen recognition in humoral antibody response. These studies provide interesting insights into physiological processes with out contradicting any rules of structural biology, the reason for the existence of dichotomy in specificity and degeneracy in the humoral response need to be addressed. With every new study, completely novel modes of binding and unexpected features continue to emerge from antigen-antibody complexes. Consolidation of these data in a coherent manner would help draw critical inferences that complete the picture and possibly provide answers for the observed shift in the paradigm in antigen specificity.

Germline gene encoded antibodies are expected to bind to the cognate antigen with high affinity and specificity after affinity maturation. Yet, no perfect apposition has been observed in the established paradigm and the vast amount of emerging data**.** It is anticipated that a coherent analysis of global data would further shed light on understanding the nature of antigen recognition. In order to dissect the apparent behaviour of the antibodies sharing common ancestor while exercising their recognition potential, we have compiled structural data on antibody-antigen complexes. Nearly 3500 antibody structures have been determined of which more than 650 structures delineate the full extent of antibody-antigen interactions. The final unique dataset is a compilation of 224 structures of which 148 mature antibodies are of mouse origin encoded by 38 different germline genes and 76 mature antibodies are of human origin encoded by 14 different germline genes. These mature antibodies that have arisen from a common germline gene, bind to antigens that are chemically distinct. Plasticity of combining site is possibly designed to disregard antigenic variations. Such an observation in mature antibodies is rather enigmatic and hence needs to be addressed holistically. It is therefore envisaged that the corresponding germline antibody should apparently recognize all of those epitopes bound by the mature antibodies. Understanding structural correlation of antigen recognition among mature antibodies is anticipated to provide information on how antigen modulates antibody evolution during affinity maturation.

Continuing the studies on structural biology of plant seed proteomics, structure of SM80.2, preliminary crystallographic studies of which were reported last year, was completed. SM80.2 shows homology with members of prolamins family that includes non-specific lipid

transfer proteins and 2S albumin family proteins. The structure was determined ab initio and refined at a resolution of 1.87Å. The final refinement cycle at present gave an overall Rcryst 25% and Rfree of 28%. Ramachandran plot shows 174 residues (98.31%) in allowed region and 2 (1.13%) in preferred region. Only one residue is present as outlier.

The overall structure of SM80.2 consists of 177 residues with continuous density. It consists of two monomers in the asymmetric unit. This high-resolution data helped in extracting the complete amino acid sequence of SM80.2. Sequence analysis of SM80.2 showed 80 % sequence similarity with non-specific lipid-transfer protein AP10-like from Solanum lycopersicum. A peculiar unexplained continuous density was also found while refinement. As this protein is a non-specific lipid transfer protein and thus is involved in transferring of lipids, we expect this continuous unexplained density may correspond to lipid moiety bound to protein. This lipid binding could be linked to allergenic activity shown by non-specific lipid transfer protein. Identification of the lipid in the observed electron density is being undertaken.

**Publications**

***Original peer-reviewed articles***


1. Jain A, Salunke DM (2015) Purification, identification and preliminary crystallographic studies of an allergenic protein from *Solanum melongena*. *Acta Cryst F Struct Biol Commun* **71**:221-225.
2. Khan T, Salunke DM (2014) Adjustable locks and flexible keys: plasticity of epitope-paratope interactions in germline antibodies. *J Immunol* **192**:5398-5405.
3. Gill J, Jayaswal P, Salunke DM (2014) Antigen exposure leads to rigidification of germline antibody combining site. *J Bioinform Comput Biol* **12**:1450006.



# Srinivasa-Gopalan Sampathkumar

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

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Our laboratory of chemical glycobiology strives to develop carbohydrate-based small molecules as probes, tools, and inhibitors to shed light on the functional roles of glycosylation in biological processes. We perform design and development of: (**A**) non-natural monosaccharide analogues for inhibition of glycosylation, which could provide valuable disease modulators for auto-immune diseases; (**B**) carbohydrate-neuroactive (CH-NA) hybrids for metabolic glycan engineering (MGE) of central nervous system (CNS) across the blood-brain barrier (BBB) to enable investigation of roles of glycosylation in CNS development and disorders; (**C**) glycopeptidomimetics (GPM) based inhibitors of matrix metalloproteinases (MMP) as anti-metastatic agents; and (**D**) inhibitors of human neuraminidases.

**A.** In continuation with our efforts on the development of inhibitors for mucin type *O*-glycosylation (MTOG), investigations on K562 (human erythroleukemia), U937 (human histiocytic leukemia), EL-4 (mouse T-lymphoma), and RAW264.7 (mouse macrophage) have been carried out. Cells were incubated with various GalNAc analogues and probed with lectins and anti-CD43 antibodies. A comparative analysis of Jurkat and K562 cells on hyposialylation and down regulation of sialoglycoforms of CD43 revealed that the differential effect of the analogues could be attributed to inherent variations in the glycosylation machinery of the cells. U937 cells exhibited strong homotypic adhesion upon treatment with $Ac_5$GalNTGc, but not $Ac_5$GalNGc and $Ac_4$GalNAc. Upon treatment with $Ac_5$GalNTGc, both EL-4 and RAW264.7 cells showed reduction in CD43-S7 and CD43-1B11 epitopes, respectively. Lectin blots of EL-4 cells revealed a reduction in *Maackia amurensis* lectin-II epitopes with a concomitant increase in *Vicia villosa* agglutinin epitopes consistent with hypo-sialylation induced by $Ac_5$GalNTGc.

**B.** Having confirmed the unique ability of CH-NA hybrids, but not parent non-hybrids, to access CNS tissues including brain across the BBB, we extended our studies. First, the *N*-azidoacetyl-D-neuraminic acid was used as an abiotic bio-orthogonal handle to indentify viable carrier molecules. Subsequently, using viable carriers, *N*-acyl-D-mannosamine derivatives were used for modulation of polysialic acidneural cell adhesion molecule (PSA-NCAM) levels in brain of living mice. Western blotting using anti-PSA and anti-NCAM molecule antibodies showed significant reduction of PSA levels on NCAM upon intravenous administration of CH-NA hybrids.

**C.** Towards GPM based MMP inhibitors; several selectively protected glycosyl donors have been synthesized. Under Königs-Knorr and Schmidt glycosidation conditions, the products were obtained in moderate yields. Preliminary cytotoxicity assays *in vitro* revealed minimal toxicity.

**D.** Towards development of NEU3 inhibitors we have established assays using NeuAc-MU as a substrate using fluorimetry. FLAG-tagged NEU3 was over-expressed in HEK293T cells, purified by immunoprecipitation, and confirmed by silver staining and western blotting.

**Publication**

***Original peer-reviewed article***

1. Golegaonkar S, Tabrez SS, Pandit A, Sethurathinam S, Jagadeeshaprasad MG, Bansode S, Sampathkumar SG, Kulkarni MJ, Mukhopadhyay A (2015) Rifampicin reduced advanced glycation end products and activates DAF-16 to increase lifespan in *Caenorhabditis elegans*. *Aging Cell* **14**:463-473.



# Kanwaljeet Kaur

## Role of carbohydrates in modulating the structure and function of glycopeptides

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**Theme of research**

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 glycopeptides.

**Objectives**

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

**Antimicrobial peptides**

Indolicidin (ILPWKWPWWPWRR-$NH_2$) which belongs to the cathelicidin family, has anti-endotoxic activity and a broad spectrum of antimicrobial activity. However, at the bactericidal concentrations, indolicidin exhibits substantial toxicity towards mammalian erythrocytes and other cells. For investigating the sugar potential in reducing its cytotoxic property and detailed structural and functional effect of glycosylation on this antibacterial peptide, we have designed and synthesized its glycosylated forms by incorporating glycosylated amino acid in place of the critical tryptophans. The designed analogues, α-GalNAc-$T^{6}$-Indolicidin and α-GalNAc-$T^{11}$-Indolicidin, were compared for their antibacterial potential, haemolytic and cytotoxic activities with that of native indolicidin. Both the glycosylated analogues displayed higher MIC in comparison to that of indolicidin. However, both the glyco-analogues were found to be non-toxic to erythrocytes and macrophage cell lines. The above results suggested that substitution of W at $6^{th}$ and $11^{th}$ positions were indeed resulting decrease in the cytotoxicity significantly but also compromising the antimicrobial potency and indicating the important role of $W^{6}$ and $W^{11}$ in the

determination of both antimicrobial and cytotoxic properties of indolicidin. The strategy for further designing of the analogue involved the substitution of $W^9$ with glycosylated threonine along with the replacement of $P^7$ to $K^7$ resulting designed peptide, α-glyco-$Thr^9K^7$-Indolicidin. The designed glycosylated analogue exhibited antimicrobial activity comparable to native indolicidin against all the tested different bacterial strains and it was found to be non-haemolytic at the concentration as high as 100M and non-toxic to macrophage cell line even at the concentration of 100μg/ml.

**Thrombin-inhibitory glycopeptides**

It was known that the C-terminal of HP6 inhibited thrombin function by interacting weakly with the exosite-1 alone. We have designed HP6 derived bivalent thrombin inhibitors, which could interact both with exosite-1 and with active site. Towards this end, we have tethered an active site binding motif (D)fPRP to the C-terminal HP6 with and without glycine linker. The non-glycosylated ($BiG_0HP6$ and $BiG_1HP6$) and glycosylated (α-$GalNAcBiG_0HP6$ and α-$GalNAcBiG_1HP6$) forms of these designed bivalent peptides were synthesized to understand the impact of glycosylation on their inhibitory efficacy. All the bivalent HP6 inhibitors exhibited inhibitory constant ($K_i$) in nano-molar range. Non-glycosylated bivalent analogue bearing one glycine residue in the linker region ($BiG_1HP6$) displayed the higher inhibitory activity than that of $BiG_0HP6$. Eventhough glycosylation was found to enhance the inhibitory potential of HP6 analogue; the same was not observed in case of bivalent HP6 inhibitors. On the contrary, α-GalNAc bearing bivalent HP6 peptides turned out to be weaker inhibitors in comparison to their corresponding non-glycosylated forms.

To get a further insight into the kinetics of association and dissociation of these inhibitors with thrombin, the surface plasmon resonance studies were carried out. The $K_D$ value obtained for glycosylated bivalent HP6 inhibitor α-$GalNAcBiG_1HP6$ was found to be higher than that of the $K_D$ value obtained for non-glycosylated analogue, $BiG_1HP6$. This observation compliments our inhibition constants ($K_i$) derived from enzyme inhibitory kinetics data.

**Publication**

***Original peer-reviewed article***

1. Lele DS, Talat S, Kumari S, Srivastava N, Kaur KJ (2015) Unerstanding the importance of glycosylated threonine and stereospecific action of Drososcin, a proline rich antimicrobial peptide. *Eur J Med Chem* **92**: 637-647.




# Vidya Raghunathan

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

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*Leishmania* species uses multiple isoforms for various enzymes of the energy pathway, one of which is phosphoglycerate kinase (PGK). These isoforms has some distinct structural features, as PGKB and PGKC differ in a handful of internal residues and in the presence of a long extension at the C-terminus of PGKC. We targeted drug development efforts, either at the glycosome itself or at the enzymes present within them for which, targeting unique structural features is critical. The enzymology and structure of PGK forms was investigated by mapping the metabolic profile of *Leishmania spp.*

The main objectives are:

1. Expression, purification, determination and steady state kinetics study of specific activities of PGKB-Lmex and PGKC-Lmex.
2. Comparison between pH optimum activity and enzyme inhibition of PGKB-Lmex and PGKC-Lmex by salt and suramin.
3. $^{31}P$ NMR studies using substrate / enzyme (PGKB-Lmex or PGKC-Lmex) mixtures, with, either no metal, $MgCl_2$, $CaCl_2$, $MnCl_2$ or $CoCl_2$ to determine the change in the dissociation constant of substrate with metal ions. The data was compared from similar experiments in literature with yeast PGK using Mg-ADP and Mg-ATP.
4. The peptides used in the Peptide based studies of glycosomal membrane association of PGKC-Lmex were used as models to understand the structural basis of the biochemical differences between PGKC-Lmex and PGKB-Lmex.
5. Conformational studies by NMR using site non-radioactive isotope labelling.
6. The promastigote and amastigote cultures of *Leishmania spp* for metabolome mapping were used. The concentration of specific metabolites in the cell at a particular time were monitored at the micromillimolar level by $^{31}P$, $^{1}H$ and $^{13}C$ NMR spectroscopy. The metabolites detected were alanine, lactate, acetate, pyruvate, succinate, glycerol, urea, $CO_2$, oxalate, valine, glutamine and arginine.

In lieu of the structure of the peptide as determined by NMR in solution [S. Kaushik, *et al.*, Mol. Biochem. Parasit. 185 (2012) 27-35], we have launched structure of the entire 62-residue C-terminal domain extension of PGKC-Lmex, cloned in *E. coli* BL21 (RP) strain. The protein expression 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.




# GENE REGULATION

# GENE REGULATION


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# Sher Ali

## Characterization of Minisatellite tagged mRNA transcripts from the human sperm genome

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Using spermatozoal cDNA from different categories of the infertile males and two minisatellite based primers 33.15 (CACCTCTCCACCTGCC) and MN3 (GAAAGAAAGAAAGAAAGAAAGAAA), we conducted minisatellite associated sequence amplification. A total of fifty one 33.15 tagged mRNA transcripts and sixty, MN3 tagged ones were uncovered. We analysed corresponding genes and detected copy number variation in the infertile males both in blood and spermatozoal samples but not in the normal fertile ones. All the transcripts showed high level of expression in spermatozoa compared to any other somatic tissues. Sperm genome analysis enabled to capture elusive gene(s) implicated in control and regulation of human male in/fertility.

***Mining of mRNA transcripts using 33.15 and MN3 mediated MASA Reaction***

Cloning and sequencing of the resultant ampliconsuncovered by MASA led to the identification of 51 different 33.15 tagged and sixty MN3 tagged mRNA transcripts in the spermatozoa from the normal, oligospermic, azoospermic and infertile males with normal spermiogram as mentioned above. The cDNA sequences were submitted to GenBank. Further, each individual sequence was used to search database to ascertain homology with the known sequences. Among 33.15 tagged transcripts, 13 showed homology with the 12 known genes in human. Others showed no homology with any of the known genes and therefore were placed in novel category.

***Expression levels of tagged mRNA transcripts in males representing different categories using Real time PCR***

The expression of 33.15 tagged transcripts was assessed across all the patients from different categories and normal fertile males using spermatozoal cDNA and transcript specific primers. The patients of a given category showed inter-individual differences in expression of repeat-tagged genes. The

expression profiles showed significant differences between normal males and that of the patients. Several transcripts expressed in all the categories of males, while others did not.

Present study provides a road map to have a comparative profile of the satellite tagged transcripts in different categories of infertile males narrowing the search of genes possibly implicated in testicular dysfunction in general and impaired spermatogenesis in particular.

**Publications**

***Original peer-reviewed articles***


1. Sharma M, Rawal L, Panwar D, Sehgal N, Ali S (2014) Differential expression of Homeobox C11 protein in water buffalo *Bubalus bubalis*and its putative 3D structure. *BMC Genomics* **15**: 638-349.
2. Panwar D, Rawal L, Ali S (2014) Molecular docking uncovers TSPY binds more efficientlywith eEF1A2 compared to eEF1A1. *J Biomol Struct Dyn* **21**: 1-12.
3. Rawal L, Pathak D, Sehgal N, Ali S (2015) Transcriptional dynamics of Homeobox C11 gene in water buffalo *Bubalus bubalis*. *DNA Cell Biol* **34**:400-411.



# Vinay Kumar Nandicoori

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

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***Protein kinase A (PknA) of Mycobacterium tuberculosis is independently activated and is critical for growth in vitro and survival of the pathogen in the host***

Protein phosphorylation events are especially known for their influence on the regulation of a number of cellular processes including gene regulation, cell growth and division. PknA and PknB are known to have profound effects on processes involved in determining cell shape and morphology, and possibly cell division. Based on transposon mutagenesis both PknA and PknB have been thought to be essential. Though PknB has been demonstrated to be essential both for *in vitro* growth and *in vivo* survival, to date there are no reports addressing the question of whether PknA is essential for growth or survival. In a recent study, we dissected the role of PknA in mediating cell survival *in vitro* as well as *in vivo*. We observed aberrant cell shape and severe growth defects when PknA was depleted (Fig. 1). Using the mouse infection model we observe that PknA is

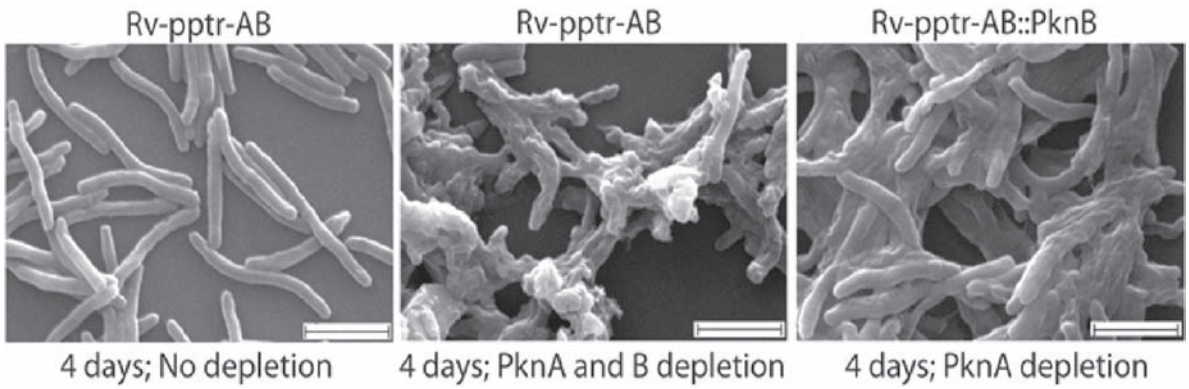



***Figure 1***: ***PknA depletion in M. tuberculosis results in aberrant cell morphology.*** Morphology of Rv-pptr-AB and Rv-pptr-AB::PknB observed through scanning electron microscopy. Rv-pptr-AB and Rv-pptr-AB::PknB cultures were seeded at $A_{600}$ of 0.1 and grown either in presence or absence of pristinamycin for 4 days. Cultures of Rv-pptr-AB::PknB were grown in presence of ATc (to provide PknB) (in the absence of inducer) for 4 days. *Scale bar*, 2 µm.

essential for survival of the pathogen in the host. Complementation studies affirm the importance of the kinase, juxtamembrane and transmembrane domains of PknA. Surprisingly, the extracytoplasmic domain is dispensable for cell growth and survival in vitro. We find that phosphorylation of the activation loop at $T_{172}$ of PknA is critical for bacterial growth. PknB has been earlier suggested to be the receptor kinase, which activates multiple kinases including PknA by trans phosphorylating their activation loop residues. Using phospho-specific PknA antibodies and conditional *PknB* mutant we find that PknA autophosphorylates its activation loop independent of PknB. Fluorescently tagged PknA and PknB show distinctive distribution patterns within the cell, suggesting that while both kinases are known to modulate cell shape and division, their modes of action are likely to be different. This is supported by our findings that expression of kinase-dead PknA versus kinase-dead PknB in mycobacterial cells leads to different cellular phenotypes. Data indicates that though PknA and PknB are expressed as part of the same operon, they appear to be regulating cellular processes through divergent signalling pathways.

***Comparative proteomic analyses of avirulent, virulent and clinical strains of Mycobacterium tuberculosis identifies strain-specific patterns***

The ability of a pathogen to survive under harsh conditions within the host is connected to its ability to modulate host cellular processes to its advantage. *Mycobacterium tuberculosis* (*Mtb*) is an adaptable intracellular pathogen, existing in both dormant as well as active disease-causing states. Quantitative protein expression profiling has proven to be a useful method in understanding how mycobacterial species adapt to different stress conditions. Here we report systematic proteomic analyses of four strains - H37Ra, H37Rv, and clinical isolates BND and JAL, to determine the differences in protein expression patterns that contribute to their virulence and drug resistance. Resolution of lysates of the four strains by liquid chromatography, coupled to mass spectrometry analysis identified a total of 2161 protein groups covering ~54% of the predicted

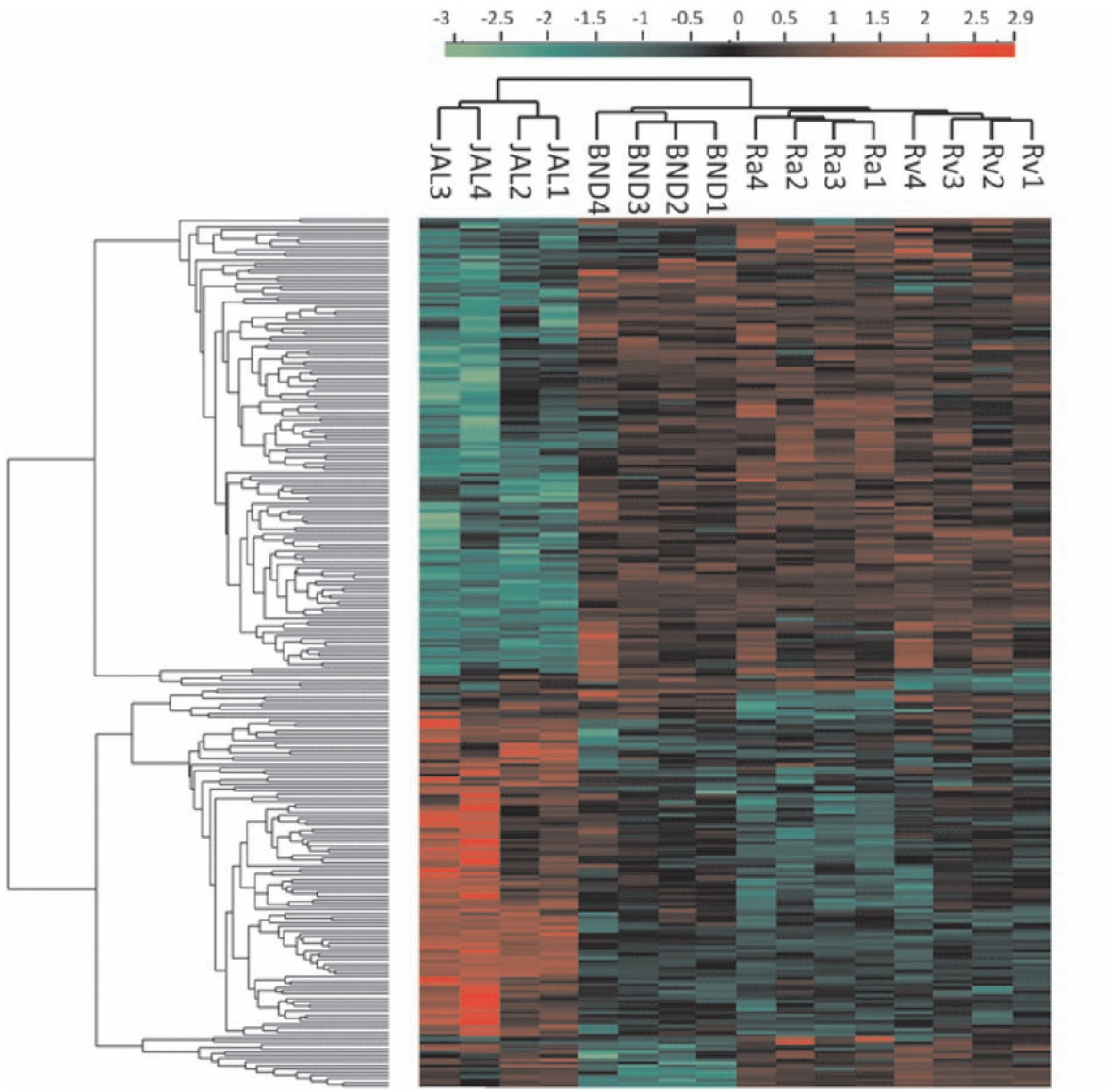



***Figure 2. Relative protein quantification.*** Clustergram of the ANOVA significant 257 proteins differing among mycobacterial strains H37Ra (Ra), H37Rv (Rv), BND and JAL strains. The Z-scored normalized abundance values of proteins are represented by red (high abundance) and green (low abundance) colors as indicated in the color scale bar at the top. The two highlighted clusters represent proteins with decreased (green) and increased (red) abundance in JAL strain.

*Mtb* proteome. Label-free quantification analysis of the data revealed 257 differentially expressed proteins (Fig. 2). The differentially expressed proteins are classified into seven K-means cluster bins, which broadly delineated strain-specific variations. Expression pattern analyses of transcription factors and their downstream targets demonstrated substantial differential modulation in JAL, suggesting a complex regulatory mechanism. Results showed distinct variations in the protein expression patterns of Esx and mce1 operon

proteins in JAL and BND strains, respectively. Lowered expression of the mce1 operon, involved in the regulation of lipid metabolism, *in BND* significantly compromised its ability to utilize cholesterol as the sole carbon source. Abrogating higher levels of ESAT6, an important Esx protein known to be critical for virulence, in JAL strain diminished its virulence, while it had marginal impact on the other strains. Taken together this study reveals that strain-specific variations in protein expression patterns have meaningful impact on the biology of the pathogen.

**Publications**

***Original peer-reviewed articles***


1. Nagarajan SN, Upadhyay S, Chawla Y, Khan S, Naz S, Subramanian J, Gandotra S, Nandicoori VK (2015) Protein kinase A (PknA) of *Mycobacterium tuberculosis* is independently activated and is critical for growth in vitro and survival of the pathogen in the host. *J Biol Chem* **290**:9626-9645.
2. Rajanala K, Sarkar A, Jhingan GD, Priyadarshini R, Jalan M, Sengupta S, Nandicoori VK (2014) Phosphorylation of nucleoporin Tpr governs its differential localization and is required for its mitotic function. *J Cell Sci* **127**:3505-3520.
3. Chawla Y, Upadhyay S, Khan S, Nagarajan SN, Forti F, Nandicoori VK (2014) Protein Kinase B (PknB) of *Mycobacterium tuberculosis* is essential for growth of the pathogen *in vitro* as well as for survival within the host. *J Biol Chem* **289**:13858 13875.



# Arnab Mukhopadhyay

## Elucidating the molecular mechanisms of aging and innate immunity using *Caenorhabditis elegans*

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We use *Caenorhabditis elegans* as a model system to understand the molecular basis of aging employing a combination of genetics, molecular biology and genomics. We are trying to understand the complex interplay of transcription factors and co-regulators downstream of the Insulin-IGF-1-like signalling (IIS) pathway in regulating longevity. Since Dietary Restriction (DR) increases life span and delays age-onset diseases, we are also trying to decipher the molecular events that follow initiation of DR.

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

We had earlier performed ChIP-seq analysis to map the genome-wide distribution of DAF-16 during normal or low insulin-IGF-1 signaling conditions. We then completed a transcriptomic analysis to reveal genes that are dependent on daf-16 when IIS is low, a condition that dramatically increases life span. We found that not all DAF-16 binding on the chromatin has transcriptional consequences. We shortlisted 37 high-confidence DAF-16 direct targets and systemically knocked them down to study their effects on *daf-2*-dependent phenotypes. We found that this set of 37 genes is required differentially for all the phenotypes of *daf-2(e1370)*. We also showed that DAF-16 predominantly acts as a transcriptional activator and binding within the 0.5 kb promoter-proximal region results in maximum induction of downstream targets that code for proteins involved in detoxification and longevity. Interestingly, genes that are activated under low IIS already have higher DAF-16 recruited to their promoters in WT. DAF-16 binds to variants of the FOXO consensus sequence in the promoter proximal regions of genes that are exclusively targeted during low IIS. Additionally, we showed that nuclear hormone receptor DAF-12 as well as zinc-finger TF EOR-1 may bind DNA in close proximity to DAF-16 and distinct TF classes that are direct targets of DAF-16 may be instrumental in regulating its indirect targets.

We have also started a systematic analysis of a zinc finger protein gene (*zfp-1*) that DAF-16/FOXO directly regulates. ZFP-1 is a

chromatin modulator and interacts with another chromatin-associated protein GFL-1. Even *gfl-1* was found to be a direct transcriptional target of DAF-16. Preliminary data suggests that ZFP-1 is regulated by multiple isoforms of DAF-16 and knocking it down affects multiple phenotypes associated with the IIS pathway.

**B. Involvement of miRNA-Transcription factor networks in dietary restriction (DR)**

We had performed miRNA-seq to identify DR-specific miRNAs. After detecting known miRNAs that are upregulated during DR, we performed novel miRNA discovery using the miRdeep2 package and validated them experimentally. Next, we performed transcriptomics analysis to compare the gene expression changes in *eat-2(ad1116)*, a genetic model of DR and compared it to wild type. We found that a large contingent of genes is upregulated on DR that are direct targets of PHA-4/FOXA. Interestingly, we found that PHA-4 directly binds to a significant number of the upregulated genes. Thus during DR, PHA-4 transcriptionally upregulates the expression of both mRNA and miRNA, forming a large network that is enriched for feed-forward motifs. This may be an efficient strategy for reducing fluctuations in gene expression during times of energy crisis. Interestingly, such networks were not found downstream of the IIS pathway, showing that these two nutrient sensing pathways function differently to control longevity.

**C. Involvement of novel kinases in dietary restriction**

We have earlier shown that following initiation of DR, there is a shift in metabolism towards fatty acid oxidation and a concurrent increase in xenobiotic genes in a PHA-4/FOXA-dependent manner. We hypothesize that xenobiotic detoxification is required to detoxify putative lipotoxins generated due to fatty acid oxidation. We found that apart from PHA-4, other transcription factors (NHR-8 and AHR-1) that are required for transcription of xenobiotic genes are also required for DR-mediated life span extension. In order to further understand how metabolic reprogramming can signal to upregulate the expression of the xenobiotic detoxification genes; we performed a targeted genetic screen. Preliminary data suggest that p38 MAPK pathway but not JNK pathway may be important for this purpose.

The *drl-2* gene was also characterized in details and was found to possess most of the phenotypes associated with *drl-1*. However, unlike *drl-1*, knocking down *drl-2* specifically in the intestine is sufficient to extend life span and decrease fat storage. We also found that the *drl-2* mutants have increased osmotolerance and we are trying to link it to the life span.

**D. Role of the Endoplasmic reticulum in DR-mediated longevity**

We have recently observed that in two independent genetic models of DR, a transient upregulation of an ER stress marker (ER chaperon HSP-4) occurs. This upregulation is dependent on the ER sensor IRE-1 and the ER stress-responsive transcription factor XBP-1. This response was specific to ER as we did not witness upregulation of mitochondrial stress marker HSP-6 or cytosolic stress marker HSP-16.2. We are now studying the functional consequences of this phenomenon on life span.

**Publications**

***Original peer-reviewed articles***


1. Golegaonkar S, Tabrez SS, Pandit A, Shalini S, JagadeeshaPrasad MG, Bansode S, Sampathkumar SG, Kulkarni MJ, Mukhopadhyay A (2015) Rifampicin reduces Advanced Glycation End products and activates DAF-16 to increase life span in *Caenorhabditis elegans*. *Aging Cell* **14**:463-473.
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3. Chamoli M, Singh A, Malik Y, Mukhopadhyay A (2014) A novel kinase regulates dietary restriction-mediated longevity in *Caenorhabditis elegans. Aging Cell* **13**: 641-655.



# Lalit C. Garg

## Molecular biology of infectious diseases

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**(A) Development of recombinant and DNA based vaccine against *Clostridium perfringens***

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. In order to develop sub-unit vaccine against beta toxin, immunodominant epitopes of the beta toxin were identified using bioinformatics tools and were cloned in translational fusion with B-subunit of heat labile enterotoxin. Recombinant fusion proteins were purified and analyzed for pentamer formation and GM1 receptor binding. Immunization of BALB/c mice with the fusion proteins generated a very good immune response. The antiserum generated against the fusion protein LTB-$Epi_{140}$significantly neutralized the toxin in an *in vitro* assay. The toxin-antisera formulations reduced cell death when compared to serum from the PBS immunized mice. *LTB-*$Epi_{140}$fusion protein-immunized mice demonstrated nearly 40 % protection against beta toxin toxicity when challenged with minimal lethal dose of beta toxin.

For the development of candidate DNA-based vaccine against beta toxin of *C. perfringens*, beta toxin gene was cloned in a mammalian expression vector for secretory expression of the recombinant protein. To investigate the immunogenic potential of this construct, mice were immunized through intramuscular route and booster doses were given 2 weeks after primary immunization. Two booster strategies i.e. homologous prime boost and heterologous prime-boost were used. Heterologous boosted mice mounted stronger antibody response against beta toxin when compared to that obtained with homologous boosted mice.

Antisera obtained from mice immunized with the DNA construct using heterologous prime boosting offered better neutralization potential than that obtained from homologous boosting. Intranasal immunization with the recombinant DNA construct was also evaluated using both homologous and heterologous prime boosting strategies. Heterologous prime boosting generated stronger immune response and showed 100% protection *in vivo* against the beta toxin challenge.

To generate non-toxic mutants for safer vaccine, and to understand the role of different residues in biological properties of the beta toxin, a large number of amino acid residues were mutated in different regions of the toxin by PCR and site-directed mutagenesis and the introduction of mutations was confirmed by DNA sequencing. The mutated genes were cloned in *E. coli* expression vector.

**(B) Studies on the functional characterization of PE_PGRS and PE_PPE proteins of *Mycobacterium tuberculosis* H37Rv**

*Mtb* employs numerous intelligent strategies to persist inside host and evade the host defense mechanisms. The knowledge of the genome sequence of *Mtb* H37Rv illuminated the existence of a unique family of genes, the PE_PGRS family, containing about 65 members. Several PE_PGRS proteins have been shown to influence mycobacterial survival inside macrophages and modulate macrophage immune functions. We examined the function of PE_PGRS35 of *M. tuberculosis* in pathogenesis and immune evasion by expressing the protein in *M. smegmatis* as this mycobacterial species naturally lacks PE_PGRS family of genes. Expression of PE_PGRS35 in *M. smegmatis* altered the growth profile of the bacterium and the recombinant protein localized in the mycobacterial cell wall. Infection of PMA-differentiated human THP-1 macrophages with *M. smegmatis expressing PE_PGRS35* led to diminished production of pro- inflammatory cytokines as compared to those infected with *M. smegmatis* alone. No change in intra-cellular bacterial survival and macrophage viability were observed.

**Publications**

***Original peer-reviewed articles***


1. Karan S, Kaushik H, Saini N, Sahoo PK, Dixit A, Garg LC (2014) Genomic cloning and sequence analysis of Interleukin-10 from *Labeorohita*. *Bioinformation* **10**:623-629.
2. Dash P, Sahoo PK, Gupta PK, Garg LC, Dixit A (2014) Immune responses and protective efficacy of recombinant outer membrane protein R (rOmpR)-based vaccine of *Aeromonas hydrophila* with a modified adjuvant formulation in rohu (*Labeo rohita*). *Fish Shellfish Immunol* **39**: 512-523.
3. Bhatia B, Ponia SS, Solanki AK, Dixit A, Garg LC (2014) Identification of glutamate ABC-Transporter component in *Clostridium perfringens* as a putative drug target. *Bioinformation* **10**: 401-405.
4. Bhatia B, Solanki AK, Kaushik H, Dixit A and Garg LC. (2014) B-cell epitope of beta toxin of *Clostridium perfringens* genetically conjugated to a carrier protein: Expression, purification and characterization of the chimeric protein. *Prot Expr Purif* **102**: 38-44.


**Patent**


1. Garg LC, Dixit A, Gopal K (2014) Recombinant non-toxic protein vaccine against *Clostridium perfringens* infection and epsilon toxin Intoxications (European application no. 11743339.1, Validated in UK, Accepted for grant on 23/09/2014).



# Madhulika Srivastava

## Epigenetic regulation of the eukaryotic genome: Role of transcriptional insulators in organizing chromatin

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The mechanisms by which *cis*-acting regulatory elements interact with each other in context of chromatin are incompletely understood even though such interactions are crucial for appropriate regulation of nuclear processes like transcription and VDJ recombination. CTCF dependent insulators play an important role in the functional organization of the mammalian genome as they can coordinate intrachromosomal and interchromosomal contacts and thus influence *cis*-DNA interactions. A large number of CTCF binding sites have been identified genome-wide suggesting their extensive involvement in governing *cis* DNA interactions among regulatory elements. Our efforts are directed towards understanding how the mammalian insulators influence chromatin domain organization and contribute to regulation of nuclear processes. A combination of genetic, molecular and biochemical approaches are being utilized for investigations.

**Objectives**

Transcription as well as RAG mediated recombination is exquisitely regulated during development at antigen receptor loci like IgH, TCR$\alpha/\delta$, TCR$\beta$ etc. This underscores the importance of appropriate enhancer-promoter interactions. Further, recombination requires physical interaction between RSS elements associated with the V, D and J 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. By exhibiting long range interactions between different types of elements, the antigen receptor loci present a useful framework to explore the nature of interactions amongst various regulatory elements. Long range interactions of chromatin have been demonstrated to be orchestrated by CTCF. Taking advantage of this, we are currently investigating the chromatin structure and organization of the wild type and genetically manipulated TCR$\beta$ loci to understand various aspects of CTCF dependent insulator function as well as of other regulatory elements important for VDJ recombination.

To understand the interactions between Eβ, PDβ1, PDβ2 and the ectopic insulator, we have extended our previous year's analysis. Appropriate conditions for 3C-qPCR (allele specific) assay have been established and the interactions between these elements in the wild type, TCR-ins and TCR-mut alleles have been examined. We observe Eβ-PDβ1 interaction to be significantly reduced in TCR-ins allele compared to the wild type and TCR-mut alleles. This suggests that an intervening insulator prevents looping of enhancers to the promoter. In context of our chromatin structure analysis reported in the previous year, our results suggest that within the framework of altered chromatin loopscape that segregates the enhancer and promoter into separate loops, insulators also interact with the enhancer and promoter as well as organize a barrier to the interactions and/or to the influence of the enhancer on the insulated domain. Consequently, they may simultaneously appear to be an enhancer decoy, promoter decoy as well as elements that prevent tracking of enhancers and/or of other epigenetic changes in case the enhancers employ these mechanisms to activate the locus.

Several CTCF binding sites have been identified at various antigen receptor loci including TCRβ locus. Our ChIP analysis indicated significant variation between the CTCF binding sites with regard to their occupancy by CTCF and in their ability to interact with each other. To investigate this intriguing aspect, we have initiated characterization of CTCF binding sites. The variation may be a consequence of DNA sequence of the core CTCF binding sites, the regions flanking them, variation in the nucleosomal structure in the vicinity of CTCF binding sites, presence of other proteins in the flanking regions etc. These variations are likely to dictate the extent of binding/eviction of CTCF, interactions amongst them, ability to act as insulators etc. and thus help to understand their relevance for recombination.

Even though the degree of binding of CTCF and cohesin to these sites does not vary substantially between Double negative (DN) T cells and ProB cells, the interactions between the CTCF binding sites were observed specifically in the DN cells suggesting that T cell lineage specific factors might contribute to the locus organization. Since Eβ is a critical regulatory element that is active specifically in the DN cells, it is possible that Eβ and/or the proteins associated with it contribute to the TCRβ locus organization and thus impact V-to-DJ recombination beyond generating the accessibility of 25kb region of DJCβ clusters. This view is negated by a recent report which demonstrated the ability of Vβ regions to interact with DJCβ regions in the absence of Eβ. The sufficiency of this interaction for V-to-DJ recombination could not be tested as absence of the enhancer precluded even D-to-J recombination. However, in TCR-ins allele, the enhancer activity is curtailed in a position dependent manner but the enhancer is active and V-to-DJ recombination operative. We have observed that functional curtailment of Eβ activity by the ectopic insulator correlates with impairment of V-to-DJ recombination in a position dependent manner. Does this suggest an involvement of Eβ in V-to-DJ recombination in some manner ? To dissect these issues, we have initiated a study to examine interaction of Vβ regions with the Eβ and DJCβ clusters in wild type TCRβ locus as well as in presence of functional or non-functional insulator.



# Pushkar Sharma

## Role of cell signaling in eukaryotic development

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We are interested in dissection of signalling 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.***

Calcium Dependent Protein Kinases (CDPKs) are major effectors of calcium signalling in Apicomplexan parasites like *Plasmodium falciparum* and *Toxoplasma gondii*. Since PfCDPK1 is essential for parasite growth, it is refractory to gene disruption. We used a parasite line in which PfCDPK1 in which the expression of PfCDPK1 could be conditionally "knocked down". Using this parasite line, we were able to demonstrate the role of PfCDPK1 in host cell invasion. To gain further insights into how it regulates this process, efforts were made to identify its substrates using quantitative proteomics. These studies lead to the identification of ~3000 phosphopeptides and several of them were differentially phosphorylated upon PfCDPK1 knock down. The phosphorylation sites on these peptides represented direct or indirect PfCDPK1 targets, which included proteins from signalling modules, IMC and other organelles. Some of these proteins were chosen for validation, which was performed by performing *in vitro*

PfCDPK1 kinase assays. Furthermore, several target phosphorylation sites were confirmed by LC-MS/MS analysis and sites directed mutagenesis. The physiological relevance of their phosphorylation by PfCDPK1 is being elucidated.

***b. Role of phosphoinositides in parasite signaling and trafficking***

We are studying PfCDPK7, which is a target of PI(4,5)P2. As indicated above, PfCDPK-KO parasites exhibit stalled TVNs and abrogated nutrient uptake. Preliminary metabolomic studies suggested significant alterations in phospholipids like phosphatidylcholine (PC) in PfCDPK7-KO parasites. Using radiolabelled choline, we could demonstrate that PC synthesis was significantly abrogated in PfCDPK7-KO parasites. Importantly, when PfCDPK7-KO parasites, which show growth defects, were cultured at high choline concentration a modest but significant reversal of growth defect was observed. Based on these results, it is likely that PfCDPK7 may contribute to parasite development by regulating phospholipid metabolism and/or trafficking.

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

We observed that miR34a, after an initial increase, was down regulated in response to prolonged treatment with neurotoxic $A\beta_{42}$. Moreover, the expression of miR-34a undergoes significant changes in the cortex of a transgenic (APP/PS1 Tg) mouse model of Alzheimer's disease (AD); a significant decrease was observed in the APP/PS1 animals, which were >12m old. Corroboratively, cyclin D1 levels were significantly higher in these situations. Since the increase in cyclin D1 results in cell cycle re-entry and neuronal apoptosis (CRNA), the role of miR34a in this process was investigated. $A\beta_{42}$ mediated CRNA was prevented by the overexpression of miR34a. Importantly, cyclin D1 overexpression neutralized the effect of miR-34a suggesting that it may prevent CRNA *via* its ability to suppress cyclin D1. The mechanisms via which miR34a is deregulated is being investigated. We found that aberrant activation of MEK-ERK pathway caused by $A\beta_{42}$ causes deregulation of miR34a, which may result in CRNA. These studies provide insights into a novel mechanism via which CRNA may be regulated.

**Publications**

***Original peer-reviewed articles***


1. Jacot D, Frénal K, Marq J, Sharma P, Soldati-Favre D (2014). Assessment of phosphorylation in Toxoplasma glideosome assembly and function. *Cell Microbiol* **16**:1518-1532.
2. Kumar P, Tripathi A, Ranjan R, Halbert J, Gilberger T, Doerig C, Sharma P* (2014). Regulation of Plasmodium falciparum Development by Calcium-dependent Protein Kinase 7 (PfCDPK7). *J Biol Chem* **289**:20386-20395.



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# Rajesh S. Gokhale

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

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**Theme of research**

Our group elucidated mechanistic and spatiotemporal coherence of cellular processes that result in two distinct pathological diseases Tuberculosis (TB) and Vitiligo. Although unrelated, both disorders involve decisive role of unusual metabolites- complex lipids with very-long branched acyl chains produced by the TB pathogen *Mycobacterium tuberculosis* (Mtb) and heteropolymeric structurally uncharacterized melanins produced by melanocytes. Both these diseases are also characterized by unpredictable disease progression profiles. TB manifests in active, latent, reactivated and dissemination phases. Vitiligo is a chronic unstable depigmenting disorder that often shows symmetry in its manifestation. While 1/6th of the Mtb genome encodes genes involved in lipid metabolism, the only well-characterized function of melanocytes is to produce melanins. Our endeavor is to understand how small molecule metabolic networks are elaborately tuned in nature and how these pathways provide distinct advantages to the specific biological system and finally their underlying implications in disease outcome.

**Objectives**

To summarize, 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 involved in melanogenesis and decode spatiotemporal coherence associated with melanocyte-keratinocyte biology

**TNF-α and hypoxia crosstalk dictate Mtb modulation of osteoclast biology**

Bacterial pathogens can habituate diverse cell types by modulating the host machinery response. Mycobacterium tuberculosis (Mtb), being an obligate intercellular pathogen, is primarily known to survive in macrophages. While Mtb pathogenesis is classically related with lungs, there is increasing number of reports of mycobacterial infections of other tissues. Presently, it is not clear how Mtb adapts to these unconventional niches. Previously we reported that Mtb could survive in the osteoclast cells. We have now delineated mechanism by which Mtb infection of macrophage precursor cells potentiates formation of giant functional osteoclasts. Our studies show that Mtb downregulates the canonical RANK signaling after the early lineage commitment and instead TNF-α level along with hypoxic response are the dominant activators of osteoclastogenesis. Interestingly, the DosR mutant of Mtb, which is known to show compromised phenotype in hypoxic conditions, display delayed osteoclast maturation kinetics. Our study reveals another fascinating example by which Mtb exploits host-derived factors in order to establish chronic infection through subversion of the protective host responses.

**Elucidation of *Cis-Trans* Isomerases from Mtb genome**

Fatty acids and lipids are often considered the major carbon source for Mtb growth during dormancy conditions. Fatty acids are classically degraded to acetate units through β-oxidation enzymes. We earlier reported that classical FadA and FadB enzymes of Mtb could not utilize oleic acid as carbon source. We show that this inability is due to the absence of cis-trans isomerase function in the FadB protein. Since crotonase fold is known to catalyze this function, we investigated in to a family of such proteins, annotated as Ech proteins in Mtb genome. Based on genetic complementation as well as biochemical enzymatic assays, we have identified several isomerases with varying catalytic activity and substrate specificity. Our study delineates a novel family of enzymes that are crucial for Mtb utilization of unsaturated fatty acids.

**Publications**

***Original peer-reviewed articles***


1. Haque AS, Patel KD, Deshmukh MV, Chhabra A, Gokhale RS, Sankaranarayanan R (2014) Delineating the reaction mechanism of reductase domains of Nonribosomal Peptide Synthetases from mycobacteria. *J Struct Biol* **187**:207-214.
2. Shukla J, Gupta R, Thakur KG, Gokhale R, Gopal B (2014) Structural basis for the redox sensitivity of the *Mycobacterium tuberculosis* SigK-RskA σ-anti-σ complex. *Acta Cryst D Biol Cryst* **70**: 1026-1036.


***Review / Proceedings***


1. Natarajan VT, Ganju P, Ramakumar A, Grover R, Gokhale RS (2014) Multifaceted pathways protect human skin from UV radiation. *Nat Chem Biol* **10**: 542-551.



# Sagar Sengupta

## Determining the signaling and repair pathways altered in human cancer

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**BLM helicase is a substrate of Fbw7α during mitosis**

To understand the cell cycle phase(s) where BLM undergoes turnover, double thymidine block of A-15 cells (BS fibroblast GM03509 cell line complemented with chromosome 15) was carried out, followed by the release of the cells in normal growth medium for defined time periods.Reciprocal immunoprecipitations demonstrate that while BLM constitutively interacts with Fbw7α and γ isoforms in all phases of the cell cycle, the association of the Fbw7 isoforms with the poly-ubiquitylated BLM occurs only in G2/M phase (9 hrs post-release). Further, in asynchronously growing cells expressing GFP-tagged BLM, the levels of BLM in the interphase cells were substantially more than in the mitotic cells. Lack of Fbw7 stabilized mitotic BLM. This effect was specific for Fbw7α isoform.

The decrease of BLM levels only by Fbw7α indicated a specific role of the N-terminal region of the E3 ligase in the degradation process. Indeed, Fbw7αΔN mutant did not decrease BLM levels. Neither Fbw7αΔF nor Fbw7αΔWD40 mutants were able to decrease BLM levels, confirming that degradation of BLM requires both these regions. The decrease in the BLM levels due to overexpression of Fbw7α was reversed by the treatment with proteasomal degradation

inhibitor MG132. *In vitro* ubiquitylation reactions reveal that recombinant BLM is poly-ubiquitylated by Fbw7α in a F-box and WD40 domain dependent manner.

**Degradation of BLM by Fbw7α depends on GSK3β and CDK2/CyclinA2 mediated phosphorylation events at Thr171 and Ser175**

The WD40 repeats in Fbw7 bind to its substrates, which have been phosphorylated in a conserved phospho-epitope, known as the phosphodegron. We found a single stretch of sequence in BLM that matched the consensus phosphodegron motif $^{169}$FV**T**PPQ**S**HF$^{177}$. We hypothesized that this putative BLM phosphodegron should also be phosphorylated by GSK3 as it matches with the GSK3 consensus (S/TxxxS/T). GSK3β-dependent phosphorylation of BLM T171A mutant was diminished during *in vitro* kinase assays. To decipher the priming kinase that phosphorylates BLM at Ser 175 (+4 position), the core phosphodegron sequence ($^{171}$**T**PPQ**S**$^{175}$) in BLM, with all validated phosphodegron sequences in Fbw7 substrates was compared. The core sequence exactly matches with one of the two phosphodegrons in Cyclin E ($^{380}$**T**PPQ**S**$^{384}$). Cyclin E Ser384 is known to be phosphorylated by Cdk2. *In vitro* kination reveals that CDK2/Cyclin A2 phosphorylates BLM at Ser175. Consequently, BLM ubiquitylation was decreased by the presence of either GSK3β inhibitor or Cdk2/Cyclin A2 inhibitor, roscovitine. Similar decrease in the levels of Fbw7α dependent BLM ubiquitylation was observed for both BLM T171A and S175A mutants.

**Chk1/Chk2 dependent mitotic BLM phosphorylation at Thr182 is a prerequisite for its degradation**

It can be hypothesized that a mitosis specific phosphorylation event maybe required, which allows BLM to be targeted by Fbw7α. An earlier study in lab had proposed that Chk1 might phosphorylate BLM at Thr182. Using recombinant proteins and *in vitro* kinase assays; we verified that both Chk1 and Chk2 kinases phosphorylate BLM at Thr182. A phosphospecific antibody which recognizes phosphorylated BLM at Thr182 (pThr182-BLM) was generated which recognized only wildtype BLM phosphorylated by enzymatically active Chk1/Chk2. Immunoprecipitation reactions carried out with pThr182-BLM antibody, indicates that it recognizes BLM *in vivo*, specifically at Thr182 residue. pThr182-BLM antibody specifically stains only the mitotic cells in celllines expressing BLM. Presence of both Chk1/Chk2 inhibitors appreciably decreased the extent of the staining detected by pThr182-BLM antibody and rescued Fbw7α dependent degradation of BLM. These results indicate that Chk1/Chk2 dependent Thr182 phosphorylation on BLM was required for its subsequent degradation during mitosis.

**Publications**

***Original peer-reviewed articles***


1. Singh M, Bansal S, Kundu S, Bhargava P, Singh A, Motiani RK, Shyam R, Sreekanth V, Sengupta S, Bajaj A (2015) Synthesis, structure-activity relationship, and mechanistic investigation of lithocholic acid amphiphiles for colon cancer therapy. *Med. Chem. Commun* **6**: 192-201.
2. Singh M, Kundu S, Reddy MA, Sreekanth V, Motiani RK, Sengupta S*****,Srivastava A*, Bajaj A* (2014). Injectable small molecule hydrogel as a potential nanocarrier for localized and sustained *in vivo* delivery of doxorubicin. *Nanoscale* **6**:12849-1255
3. Rajanala K, Sarkar A, Jhingan GD, Priyadarshini R, Jalan M, Sengupta S, Nandicoori VK. (2014). Phosphorylation of nucleoporin Tpr governs its differential localization and is required for its mitotic functions. *J Cell Sci*. **127**: 3505-3520.



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# Sandeep Saxena

## Understanding the regulation of DNA replication

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DNA replication is a vital process of life and must be completed precisely during each cell cycle. When mammalian cell undergo DNA damage, it activates checkpoint mechanisms to stall the progression of cell cycle and DNA replication. Our laboratory is working in direction of understanding the mechanisms by which microRNAs and checkpoint proteins stall the cell cycle, preventing genomic instability and cancer. We are studying the regulation of replication machinery during stress in order to identify underlying mechanisms responsible for inhibition of essential replication factors during stress. We are trying to understand the role of ubiquitination machinery in regulating replication proteins under normal and stressed conditions. We are also investigating the cellular response to aberrations in replication complexes. The objective is to identify yet unknown checkpoint pathways that monitor the replication apparatus. Emerging evidences suggest that microRNAs target genes that regulate DNA replication and cell cycle progression and we aim to determine the role of microRNA in regulating the DNA replication machinery as the cell progresses from one phase to the next. This would provide an insight into the mechanisms by which microRNAs regulate mammalian cell cycle and DNA replication during normal and pathological conditions. Summing up, we are attempting to unravel the protective regulatory control of mammalian cells, failure of which is likely to cause genomic instability.

We have identified a novel role of alternate single-stranded DNA-binding proteins in checkpoint signaling: In summation, we report that the single-stranded DNA-binding protein complex, hSSB1-INTS3 can recruit the checkpoint complex to initiate ATR signaling. We have shown that GINS subunit is required for centrosome integrity during mitosis: We have previously identified that depletion of a GINS subunit results in multipolar spindle formation and increased centrosome number in mitotic cells, indicating that it is required for mitotic progression. We have shown a novel

role of non-coding RNAs in regulation of cell cycle: We have analyzed the role of non-coding RNAs in regulating the transition between proliferation and quiescence. We standardized two microRNA assays in our laboratory: hemi-nested and poly-A-tailing assay and utilized them to confirm the levels of microRNAs in asynchronous growing and serum starved cells.

Based on our results, we had predicted that microRNAs regulate the cell cycle genes during transition between proliferation and quiescence. Summing up, we are trying to understand the mechanisms by which microRNAs regulate mammalian cell cycle and DNA replication in normal and pathological conditions.

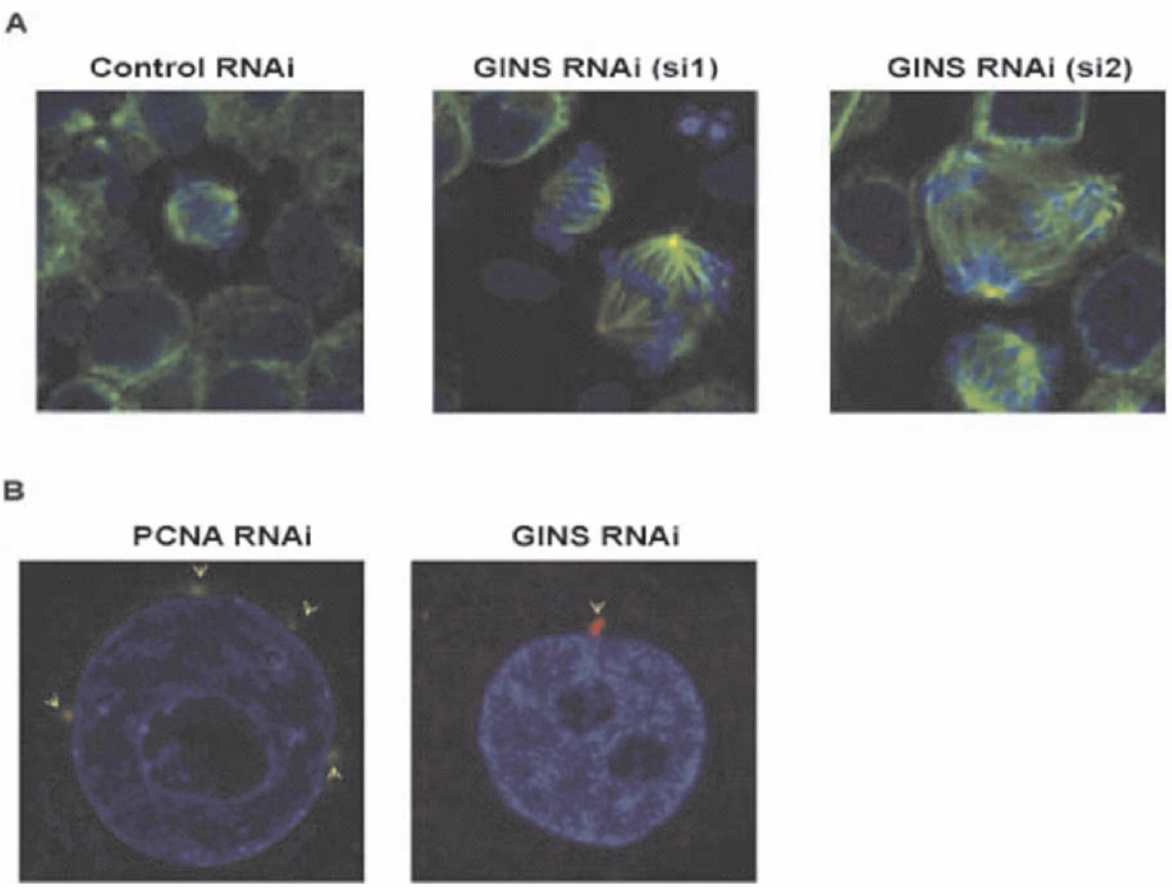



**Figure 1.** GINS depletion results in mitotic aberrations. (A) HeLa cells were transfected with control GL2 or two different GINS siRNA (si or si2) followed by co-immunofluorescence with mouse anti-alpha tubulin and rabbit anti-gamma tubulin antibodies in combination with anti-mouse Alexa Fluor 488 and anti-rabbit Alexa Fluor 555 antibodies respectively. Chromosomes were stained with DAPI. (B) HeLa cells were transfected with PCNA or GINS siRNA followed by co-immunofluorescence with mouse anti-alpha tubulin and rabbit anti-gamma tubulin antibodies in combination with anti-mouse Alexa Fluor 488 and anti-rabbit Alexa Fluor 555 antibodies respectively. Nuclei were stained with DAPI. Arrows mark the centrosomes as identified by gamma-tubulin staining. Note that mitotic aberrations are observed in PCNA depleted but not in GINS depleted interphase cells.

A

| Relative Levels by RT-PCR | | 0.01 | 0.01 | 0.01 | 0.24 | 0.02 | 0.06 | 0.07 | 0.5 | 0.08 | 0.28 | 0.04 | 0.13 | 0.64 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Relative Levels by RT-PCR | microRNA | hsa-miR-424 | hsa-miR-1 | hsa-miR-224 | hsa-miR-628-5p | hsa-miR-503 | hsa-miR-363 | hsa-miR-143 | hsa-miR-9* | hsa-miR-331-3p | hsa-miR-598 | hsa-miR-181a* | hsa-miR-10b | hsa-miR-34c-5p |
| | GENE | | | | | | | | | | | | | |
| 10.09 | CDK6 | | • | • | • | • | • | • | | • | • | | • | • |
| 8.63 | LIG1 | | | | | | | | | • | | | • | |
| 2.84 | CCNG1 | • | | | | • | • | | | | | | | • |
| 2.68 | CDT1 | • | | • | | • | • | • | | | | | | |
| 2.57 | TOPBP1 | | | | • | | • | | • | | | | | |
| 2.51 | POLE | • | | | | • | | | | | | | | |
| 515.60 | CCND2 | • | • | • | • | • | • | • | • | • | | • | • | • |
| 2.06 | MCM8 | | | | | | | • | | | | | • | |

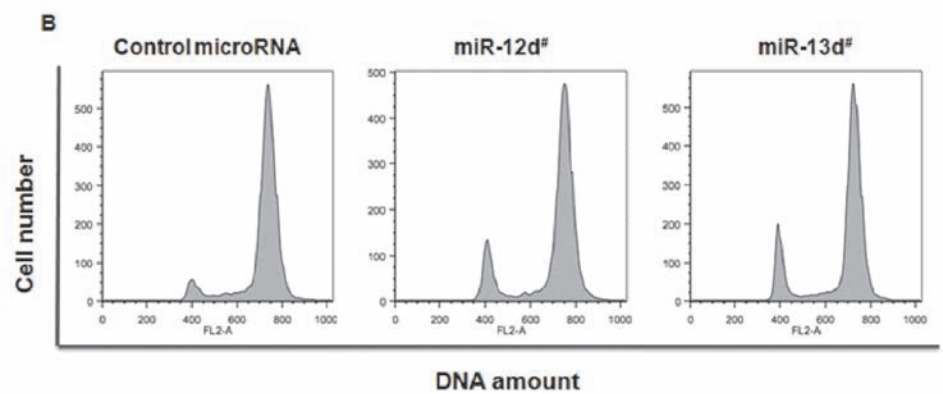



**Figure 2.** MicroRNA control of cell cycle in osteosarcoma. (A) The table displays example of cell cycle genes that are deregulated in osteosarcoma. Based on TargetScan, miRWalk and miRanda, the microRNAs that target these genes are predicted. Relative levels indicate the levels of mRNA and microRNAs in cell lines displaying aggressive osteosarcoma phenotypes in comparison to slow growing control osteosarcoma cell lines. (B) Flow cytometry of propidium iodide stained DNA from HeLa cells demonstrates a G1 block after overexpression of specific microRNAs. Cells were transfected with microRNA mimics ($^{\#}$ referred as per DRCC laboratory index) followed by treatment with nocodazole (which blocks in G2/M phase).



# Sanjeev Das

## The role of tumor suppressors in stress response

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p73 is one of the tumor suppressors of the p53 family of nuclear transcription factors. However, the molecular mechanisms underlying p73 regulation remain unanswered. To address the lacunae in the understanding of p73 stability and function, we carried out a proteomics screen to identify p73 interacting proteins under normal and genotoxic stress conditions. We have now identified TRIM28 and MED15 as potential p73 interacting proteins. TRIM28 has been shown to exhibit E3 ubiquitin ligase activity. Our results indicate that coiled coil domain of TRIM28 binds to the N-terminal transactivation domain of p73. Our results show that TRIM28-p73 interaction is curtailed with increased duration of genotoxic stress and is completely abolished at late time points due to tyrosine kinase c-abl-mediated phosphorylation of p73 at Tyrosine 99 residue. MED15 was found to be an integral component of p73-mediated transcription.

The sirtuins are highly conserved enzymes that utilize $NAD^+$ to modify other proteins. Of the mammalian sirtuins, SIRT6 recapitulates many of the biological functions of founder member of the sirtuin family, yeast Sir2. At the molecular level, SIRT6 regulates the expression of a large number of stress-responsive and metabolism related genes, promotes genomic stability and DNA repair. Since SIRT6-deficient mice exhibit profound metabolic defects, which are thought to be a primary contributor to their early lethality, understanding the role of SIRT6 in metabolic homeostasis is an area of notable interest. To gain mechanistic insights into SIRT6 regulation, we first tested the effect of different forms of metabolic stress including glucose starvation, serum starvation and cold shock, on SIRT6 levels. We found that SIRT6 protein levels were induced upon metabolic stress but there was no change in the transcript levels. We found out that there was robust induction of the SIRT6 protein levels in presence of MG132 while there was no change in the transcript levels. We then checked the SIRT6 protein for polyubiquitylation and found that SIRT6 protein

was indeed undergoing K48-linked polyubiquitylation under normal conditions, which was abrogated upon starvation. These results suggest that SIRT6 levels are regulated by ubiquitin proteasome system. To understand the complexity of SIRT6 interactome, we also carried out a LC-MS/MS based proteomics screen to identify SIRT6 interacting proteins. The result of LC-MS/MS screen identified several novel interacting proteins including Ubiquitin-protein ligase E3A (UBE3A) and Pyruvate Kinase M2. UBE3A functions as an E3 ligase in the ubiquitin proteasome pathway. The M2 splice isoform of pyruvate kinase (PKM2), is an enzyme that catalyzes the final step of glycolysis- the conversion of phosphoenolpyruvate to pyruvate. PKM2 levels are increased in human cancer tissues. PKM2 can undergo nuclear translocation by virtue of its nuclear localization signal in the C-terminal domain. In the nucleus, PKM2 activates transcription of various genes by interacting with and phosphorylating specific nuclear proteins, endowing cancer cells with a survival and growth advantage. The role of UBE3A and PKM2 in SIRT6 function and regulation is under further investigation.

**Publication**

***Original peer-reviewed article***


1. Satija YK, Das S (2015) Tyr99 phosphorylation determines the regulatory milieu of tumor suppressor p73. *Oncogene* DOI:10.1038/onc.2015.111


***Reviews***


1. Kumari R, Sen N, Das S (2014) Tumour suppressor p53: understanding the molecular mechanisms inherent to cancer. *Cur Sci* **107**:786-794.
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# ANCILLARY RESEARCH

Subeer S. Majumdar

## Production of transgenic and other animal models for biomedical research

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

We are also working to develop new easier techniques for making transgenic animals, using genes relevant to human health and diseases. One of the objectives of developing easy techniques is to use this technology for making large animals expressing therapeutic products in their milk for increasing affordability of such therapeutics, by masses.

Several disorders can be efficiently and meaningfully studied only in rat models, which are 10-12 times bigger in size than mice. However, rat transgenesis is very difficult. Hence, we established technique of testicular transgenesis for rats.

Although promoters of milk proteins from cow and goat have been isolated by Western countries, buffalo that serves as the second largest species contributing to milk production, worldwide, has not been exploited yet. To this end, β-casein genomic region which contains promoter region, exon1, intron1 and exon2 (BuCSN2, NCBI Accession No. KF612339) was isolated by us from the genome of Indian river buffalo (*Bubalus bubalis*). Coding sequences for EGFP and human Interferon-γ were cloned

downstream of this promoter. Transfection of mammary epithelial cells with this construct showed that the promoter is capable of driving non- mammalian and mammalian genes specifically in the mammary cells. ELISA of cell lysates showed expression of human interferon-γ by mammary cells.

rete testis. SSC colonized the basement membrane of the tubules after 2 months as revealed by GFP expression by donor SSC in recipient testis. The Gc transplanted testis was larger than the control testis of other age matched animal, treated with busulfan but not transplanted with the Gc. Further work is in progress.

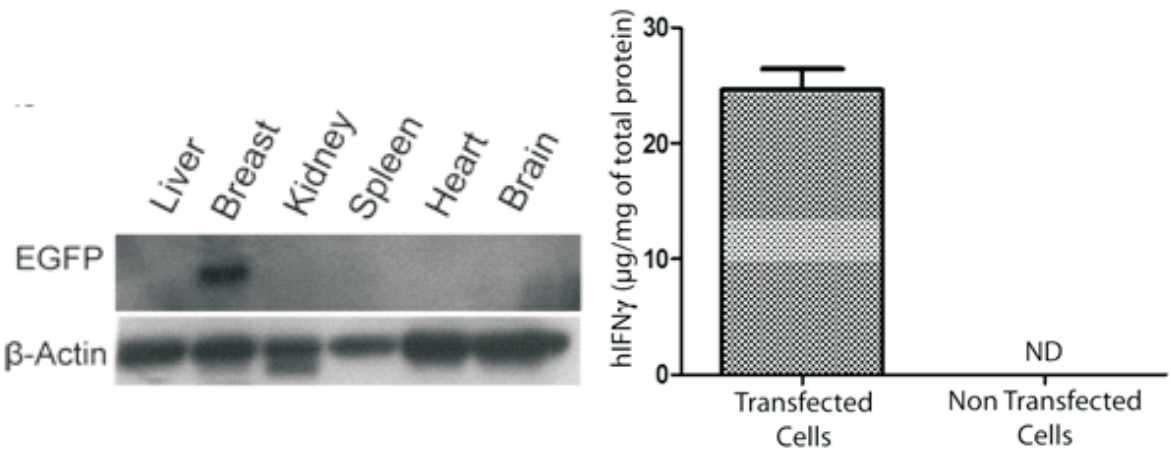



**Figure1. Left Panel:** Western Blot analysis of protein from various organs of BuCSN2-**IRES2-**EGFP transgenic mice showing mammary gland specific expression of EGFP (transgene) due to regulation of the gene by Buffalo beta casein promoter in mammary epithelial cells. **Right Panel:** BuCSN2-hIFNγ-IRES2-EGFP transfected MCF7 (human breast carcinoma cells) expressing hIFNγ, as detected by ELISA.

We reported about development of a procedure where testis can be depleted of Gc by direct injection of busulfan in the testis to create a model of infertility, post chemotherapy. The standardized dose resulted in reduction of the testis size and killing of most of the germ cells of testis within 12-15 days post injection as opposed to about 70 days required for this effect by traditionally used intraperitoneal injections. This procedure helped in preparing animal model with less pain and suffering to animal while objective of germ cell depletion was achieved. GFP expressing SSC cultured *in- vitro* were microinjected into the seminiferous tubules of such germ cell depleted respective testes (autologus transplantation) of recipient male through an efferent duct into

**Publications**

***Original peer-reviewed articles***


1. Chemmannur SV, Badhwar AJ, Mirlekar B, Malonia SK, Gupta M, Wadhwa N, Bopanna R, Mabalirajan U, Majumdar S, Ghosh B, Chattopadhyay S (2015) Nuclear matrix binding protein SMAR1 regulates T cell differentiation and allergic airway disease. *Mucosal Immunol* **8**: 1201-1211.
2. Ganguli N, Ganguli N, Usmani A, Majumdar SS (2015) Isolation and functional characterization of buffalo (*Bubalus bubalis*) β-casein promoter for driving mammary epithelial cell-specific gene expression. *J Biotechnol* **198**:53-59.

# PUBLICATIONS, PATENTS AND TECHNOLOGY TRANSFER

***A. ORIGINAL PEER-REVIEWED ARTICLES***


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***B. REVIEWS / PROCEEDINGS***


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***C. PATENTS/ TECHNOLOGY TRANSFER***

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2. Panda AK and Prasad A (2015) A method for efficient entrapment of water soluble antibiotics in polymer scaffold to be used for wound dressing
   (*Indian patent application 144/DEL/2015 filed on 16/01/2015)*

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4. Garg LC, Dixit A and Keshav G (2015) A recombinant vaccine against *Clostridium Perfringens* infection and Epsilon (ε) toxin intoxication
   *(European patent no. 2591108 granted on 11/03/2015; validated in UK)*

5. Panda AK, Gopimohan R and Anish CK (2014) Biodegradable polymer scaffold and process for preparation thereof
   *(European patent no.08842301.7 granted on 19/12/2014; validated in the UK)*

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   *(Australia patent no. 2008315318 granted on 24/05/2014*)

7. Panda AK, Singh MS, and Updhayay AK (2014) Process for obtaining bioactive recombinant protein from inclusion bodies
   *(Australia patent no. 2008249543 granted on 05/06/2014*)

8. Surolia A, Gupta S, Singh MP and Chattopadhyay T (2014) Process for preparing supramolecular calcitonin assemblies (SCA)
   *(European patent no.EP2282763 granted on 24/10/2014)*

9. Surolia A, Gupta S, Singh MP and Chattopadhyay T (2014) Composition useful for treatment of diabetes & disorder
   *(Australia patent no. 2008354530 granted on 12/06/2014)*

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    *(Australia patent no.2009200906 granted on 17/07/2014*)



# AWARDS AND DISTINCTIONS

**Dr. Chandrima Shaha** received the Om Prakash Bhasin Award and 14th Pushpa Sriramachari Foundation Day Oration Award, ICMR for the year 2014. She was also elected Fellow of The World Academy of Sciences (TWAS).

**Dr. Rajni Rani** received the Senior Scientist Oration Award from the Indian Immunology Society for outstanding and significant contributions during Immunocon 2014, held at at MKU, Madurai from 12th -14th December 2014.

**Dr. Satish K. Gupta** received Prof. L. S. Ramaswami Memorial Oration Award-2015, from Indian Society for Study Reproduction and Fertility, India.

**Dr. Sanjeev Das** received the NASI-SCOPUS Young Scientist Award-2014 in Biological Sciences, from National Academy of Sciences, India and Elsevier.

**Dr. Sarika Gupta** received Indian Academy of Biomedical Sciences Smt. Kusum Sharma Award, for outstanding contribution in biomedical research 2014.

**Dr. Anil Suri** was awarded Dr. T. C. Anand Kumar Memorial Oration Award-2015 conferred by ISSRF for the role of SPAG9 in reproductive tract cancers during International Conference on Reproductive Health 14-17 February 2015 at National Institute for Research in Reproductive Health, Mumbai, India. He also received Citation Award for 6th Professor Rabindra Nath Chakravarti- 2014 conferred by Post Graduate Institute of Medical Education & Research, Chandigarh in recognition of outstanding contribution in the field of Cancer research.

**Dr. Apurba K Sau** was the recipient of National Bioscience Award for Career Development- 2013 in June 2014.

**Dr. Nirmala Jagadish** was awarded the Prof. G. P. Talwar Gold medal for Middle Career Scientist Award-2015 conferred by ISSRF for SPAG9 in Breast carcinoma during International Conference on Reproductive Health 14-17 February 2015, at National Institute for Research in Reproductive Health, Mumbai, India.

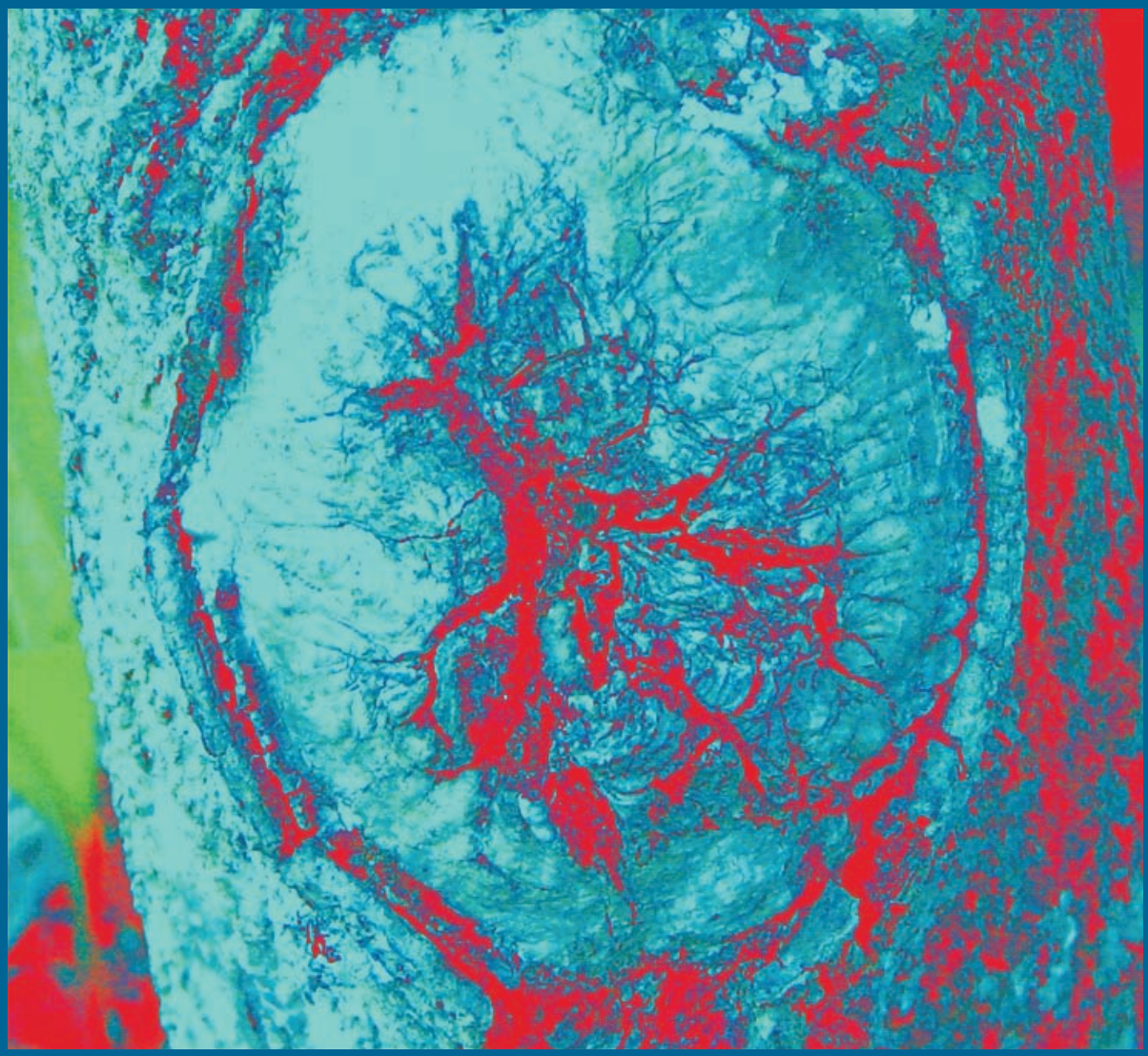
**Dr. Deepak Parashar** received the Prof. N. R. Moudgal Young Scientist Award 2015 conferred by ISSRF for SPAG9 in Ovarian Cancer during International Conference on Reproductive Health 14-17 February 2015, at National Institute for Research in Reproductive Health, Mumbai, India.

# Ph.D Degrees Awarded To NII Scholars

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

| Student's Name | Topic of Research | Guide |
|---|---|---|
| Mr. Vineet Kumar Gupta | Elucidating the role of PE and PPE proteins of *Mycobacterium tuberculosis* H37RV | Dr. Lalit C. Garg |
| Ms. Sakshi Arora | HIV-1 mediated modulation of host ubiqutin machinery in viral pathogenesis | Dr. Akhil C. Banerjea |
| Mr. Ashish Kumar | Structural proteomics of seed proteins associated with regulatory functions | Dr. Dinakar M. Salunke |
| Mr. Pawan Kumar | Evaluation of immunotherapeutic potential of *Mycobacterium indicus pranii* and underlying mechanisms in Mouse Tumor Model | Dr. Sangeeta Bhaskar |
| Mr. Sandeep Kumar Upadhyay | Elucidating the role of Serine/Threonine protein Kinase G in growth and survival of *Mycobacterium tuberculosis* inside the host | Dr. Vinay K. Nandicoori |
| Mr. Ambrish Kumar | Structure function relationship studies of the acy1 carrier protein (ACP) of *Leishmania major* | Dr. Monica Sundd |
| Mr. Sandeep Kumar Yadav | Molecular characterization of repeat tagged transcripts from the normal human spermatozoa their organizational variation and relative expression across the human cell line | Dr. Sher Ali |
| Mr. Ankur Singh Saini | Factors governing generation of long-term humoral immunity | Dr. Anna George |
| Mr. Jashdeep Bhattacharjee | Generation of hepatocyte-like cells from human blood derived monocytes | Dr. Pramod K. Upadhyay |
| Ms. Bharti Bhatia | Translational fusions of beta toxin epitopes with LTB as vaccine candidate and structure-function analysis | Dr. Lalit C. Garg |
| Ms. Nidhi Jain | Studies of infection and inflammation in immune system | Dr. Vineeta Bal |

| Student's Name | Topic of Research | Guide |
|---|---|---|
| Ms. Parul Ganju | Understanding mechanism involved in regulating skin pigmentation | Dr. Rajesh S. Gokhale |
| Mr. Prasad S. Admane | Cell growth and wound healing potential of polymeric scaffolds | Dr. Amulya K. Panda |
| Ms. Shweta Pasi | Development of Novel Protein Therapeutics for Rheumatoid Arthritis (RA) | Dr. Monica Sundd |
| Ms. Ananya Kar | Identifying the role of mammalian proteins in checkpoint activation | Dr. Sandeep Saxena |
| Mr. Rajiv Ranjan Singh | Mechanistic studies and inhibitor design for Transthyretin Amyloidosis | Dr. Sarika Gupta |
| Mr. Santosh Kumar Yadav | Antiviral and cytotoxic potential of ribotoxins, ribosome inactivating proteins and human ribonucleases: a comparative study | Dr. Janendra K. Batra |
| Ms. Nazia Nasir | Crystal structures and biochemical properties of *Mycobacterium tuberculosis* Rv 1600 and Rv 3772 encoded aminotransferases reveal the molecular bases of their substrate specificities | Dr. Bichitra K. Biswal |
| Mr. Asif S | Application of Metabolic Glycan Engineering (MGE) to neurobiology using Carbohydrate-Neuractive (CH-NA) hybrid molecules | Dr. Srinivasa-Gopalan Sampathkumar |
| Ms. Sonia Jain | Investigations into the immunobiology of hemoglobin | Dr. Rahul Pal |
| Mr. Kharat Suhas Sampat | Investingating factors affecting stability of tumor suppressors BLM helicase and p53 | Dr. Sagar Sengupta |
| Mr. Pratishtha Rawat | Functional analysis of CTCF binding at TCRb locus | Dr. Madhulika Srivastava |
| Ms. Deepika Gupta | Mechanism of regulation of Rab5 and Rab7 expression by extracellular ligands | Dr. Amitabha Mukhopadhyay |
| Ms. Shalini Tanwar | Studies in B Cell development and maturation | Dr. Satyajit Rath |
| Mr. Aich Abhishek Subhas | Functional analysis of Mitochondrial Tryparedoxin Peroxidase in *Leishmania spp* | Dr. Chandrima Shaha |

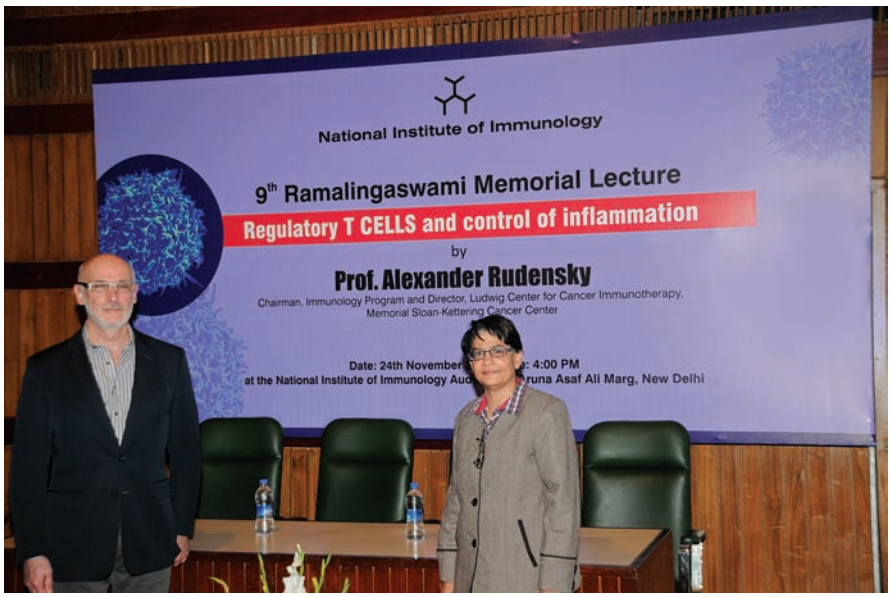


# LECTURES AND SEMINARS

**Dr. Michael L. Klein**, Dean, College of Science and Technology, Temple University, Philadelphia, USA delivered a Colloquium lecture on **"The Spark of Life : Understanding Nature's Ion Channel Design via Computation"** at the Institute on 12th January, 2015.

## RAMALINGASWAMI MEMORIAL LECTURE

Prof. Ramalingaswami, a pioneer and visionary who laid the foundations of Biomedical Research in India, also was one of the founding members of NII. The RAMALINGASWAMI MEMORIAL LECTURE, named after him and awarded each year to a distinguished scientist. The ninth Ramalingaswami Memorial Lecture was organized at the Institute on 24th November 2014. On this occasion, **Prof. Alexander Rudensky**, Chairman, Immunology Program and Director, Ludwig Center for Cancer Immunotherapy, Memorial Sloan-Kettering Cancer Center, New York delivered the lecture entitled **"Regulatory T CELLS and control of inflammation"**.




*Prof. Alexander Rudensky with Dr. Chandrima Shaha during Ramalingaswami Memorial Lecture*

## FOUNDATION DAY LECTURE

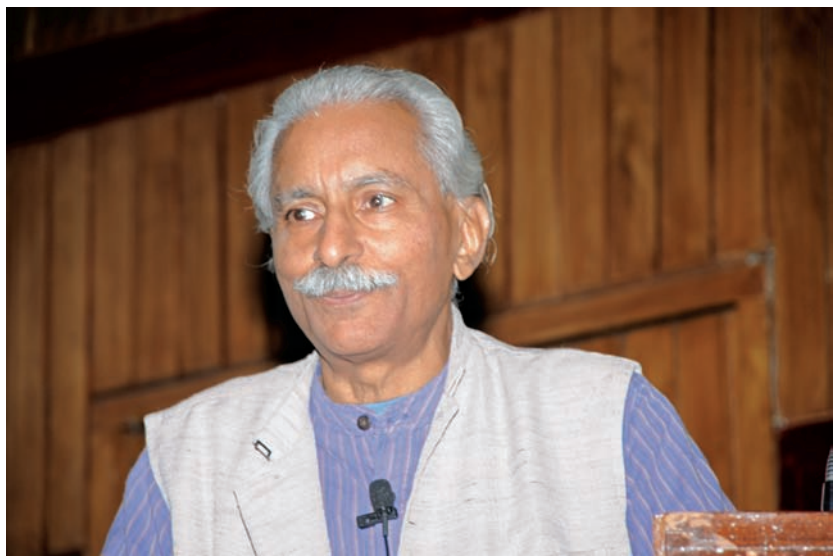
On 6th October 2014, the 28th Foundation Day of NII was celebrated. **Prof. Ajay Sood**, Indian Institute of Science, Bangalore was invited as a Guest of Honour. He delivered a lecture on **"Driven and active soft matters: Fun Science".**




*Prof. Ajay Sood after the Foundation Day Lecture*

## SCIENCE DAY LECTURE

National Science Day was celebrated on 28th February 2015 at the Institute. **Prof. Vidyanand Nanjundiah**, Centre for Human Genetics Bangalore delivered a lecture on **"Information transfer and the autonomy of biology"**.



*Prof. Vidyanand Nanjundiah delivering the National Science Day Lecture*

## PUBLIC LECTURE

**Prof. Christian Bréchot**, President, Institut Pasteur, Paris, delivered a Lecture at the Institute on "**Institut Pasteur: Novel Scientific and Strategic Challenges"** on 30th May, 2014.



*Prof. Christian Bréchot after his lecture with Dr. Chandrima Shaha*

## SPECIAL LECTURE

**Prof. Philippe J. Sansonetti**, head of the Unité de Pathogénie Microbienne Moléculaire at the Pasteur Institute of Paris and Chair of Microbiology and Infectious Diseases at the Collège de France, delivered a Special Lecture at the Institute on **"Pathogenesis of *Shigella* infection, the Yin and Yang of innate immunity"** on 13th January, 2015**.**




*Prof. Philippe J. Sansonetti delivering the lecture at NII Auditorium.*

# SEMINARS

| Sl. No. | Topic | Presented by | Date |
|---|---|---|---|
| 1. | Adaptation to Chromosome damage and some translational tales | **Dr. Uttam Surana**<br>A Star Biopolis, Singapore | 9th April, 2014 |
| 2. | How do the Structure and Dynamics of Proteins Govern their Function? | **Dr. Usha Dnadamuni**<br>The Hebrew University of Jerusalem, Israel | 17th April, 2014 |
| 3. | Signals and molecular arsenals involved in red blood cell entry and exit by the human malaria parasite | **Dr. Shailja Singh**<br>Shiv Nadar University, Uttar Pradesh, India | 22nd April, 2014 |
| 4. | Non-synaptic mechanisms of neuronal plasticity in auditory brainstem | **Dr. Sukant Khurana**<br>Cold Spring Harbor Laboratory, New York, USA | 30th April, 2014 |
| 5. | Becoming an Organelle donor: The healing touch of mesenchymal Stem Cells | **Dr. Anurag Agrawal**<br>IGIB, New Delhi | 7th May, 2014 |
| 6. | Life-altering Decisions: To renew, commit or change fate | **Dr. Suchitra Devi Gopinath**<br>Stanford University, USA | 4th June, 2014 |
| 7. | Chromatin domains and regulation of transcription | **Dr. Suresh Cuddapah**<br>NYU Langone Medical Centre, USA | 26th June, 2014 |
| 8. | How micro RNAs calibrate the immune response to *Mycobacterium tuberculosis* infection | **Prof. Joyoti Basu**<br>Department of Chemistry<br>Bose Institute<br>Kolkata | 28th July, 2014 |
| 9. | Glycomics-Applications and advances in human healthcare | **Prof. Lokesh Joshi**<br>Strokes Professor of Glycosciences,<br>Vice President for Research,<br>National University of Ireland, Galway, Ireland | 5th August, 2014 |
| 10. | MHC class I assembly and polymorphisms | **Prof. Malini Raghavan**<br>University of Michigan, Ann Arbor, USA | 6th August, 2014 |
| 11. | Translation beyond the stop codon: generation of anti-angiogenic VEGF-A isoforms | **Dr. Sandeepa Eswarappa**,<br>Cleveland Clinic, USA | 21st August, 2014 |

| Sl. No. | Topic | Presented by | Date |
|---|---|---|---|
| 12. | Membrane Protein Biogenesis: Topogenesis Assembly and Function | **Dr. Prasanna Devaraneni**<br>Oregon Health and Sciences University, USA | 3rd September, 2014 |
| 13. | Brain-wide plasticity after spinal cord injuries-A bottom up revolution | **Dr. Neeraj Jain**<br>National Brain Research centre, Manesar, Gurgaon | 4th September, 2014 |
| 14. | Towards simulating virtual Cells: The Kinesin microtubule molecular motor | **Dr. Srayanta Mukherjee**<br>Center for study of systems Biology, Georgia Institute of Technology, Atlanta GA, USA | 18th September, 2014 |
| 15. | New collaborations of formins in actin assembly and organization | **Dr. Richa Jaiswal**<br>Braneis University, USA | 13th October, 2014 |
| 16. | Exploring Phagocytosis in protozoan parasite *Entamoeba histolytica* | **Dr. Somlata**<br>JNU, New Delhi | 20th October, 2014 |
| 17. | Role of transcriptional enhancers and their hierarchical networks in gene regulation | **Dr. Dimple Notani**<br>School of Medicine UCSD, La Jolla California, USA | 31st October, 2014 |
| 18. | T cell receptor repertoire plasticity in peripheral CD8 T cell | **Dr. Pradyot Das**<br>St. Jude Children's Research Hospital, Memphis Tennessee, USA | 3rd November, 2014 |
| 19. | Burying the Dead:Mechanism, defects and diseases | **Dr. Manikandan Subramanian**<br>Columbia University Medical Center, New York, USA | 10th November, 2014 |
| 20. | Switch from extrinsic to Intrinsic Mode of Activity Control in IKK2/ beta: A new paradigm in Protein Kinase Regulation | **Dr. Smarajit Polley**<br>Department of Chemistry & Biochemistry, University of California, San Diego, USA | 19th November, 2014 |
| 21. | Epigenetic activation and oncogenic function of POTE genes in human epithelial ovarian cancer | **Dr. Ashok Sharma**<br>Nebraska Medical Center, Omaha, USA | 1st December, 2014 |
| 22. | Signal transduction in T cells: All roads lead to receptor clustering | **Dr. Rajat Verma**<br>T-Cell Biophysics Unit, Laboratory of Systems Biology, NIH, USA | 2nd December, 2014 |
| 23. | Induction and Suppression of Innate Immunity in Plant-Pathogen Interactions | **Dr. Ramesh Sonti**<br>CCMB, Hyderabad | 3rd December, 2014 |

| Sl. No. | Topic | Presented by | Date |
|---|---|---|---|
| 24. | The molecular role of X-Linked mental retardation gene CASK in brain development | **Dr. Konark Mukherjee**<br>Assistant Professor, Department of Biological Sciences, Virginia Tech, Roanoke VA24016 USA | 4th December, 2014 |
| 25. | IRAK1 as a bypass switch in multi-ligand signaling by TLRs | **Dr. Sharat Jacob Vayttaden**<br>National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA | 6th January, 2015 |
| 26. | BRD4 in Gene-Specific Targeting and Cancer therapeutics | **Dr. Chen-Ming Chiang**<br>University of Texas, Southwestern Medical Center, TX, USA | 21st January, 2015 |
| 27. | Molecular and Genetics Dissection of skeletal Physciology | **Dr. Vijay Yadav**<br>Department of Mouse & Zebrafish Genetics, Wellcome Trust Sanger Institute, UK | 22nd January, 2015 |
| 28. | Mathematical immunology for Immunologist! | **Prof. Carmen Molina-Paris**<br>Department of Applied Mathematics, University of Leeds, UK | 28th January, 2015 |
| 29. | Understanding haematopoiesis at the single cell level at WEHI | **Dr. Shalin Naik**<br>WEHI, Melbourne, Australia | 28th January, 2015 |
| 30. | A Materno-fetal approach to induce T Cell tolerance FcRn as a Trojan Horse | **Dr. Nimesh Gupta**<br>Cordeliers Research Center, Pierre and Marie Curie University, Paris, France | 5th March, 2015 |
| 31. | Kinomics of malaria parasite and its erythrocyte host | **Prof. Christian Doerig**<br>Monarsh University, Australia | 19th March, 2015 |
| 32. | Role of Human DNA Helicases in Age-Associated Human Diseases and Cancer | **Dr. Sanjay Bharti**<br>Laboratory of Molecular Gerontology, National Institute of Aging, National Institutes of Health,USA | 24th March, 2015 |
| 33. | Open problems of the Germinal Center Reaction | **Prof. Jose Faro**<br>Department of Biochemistry, Genetics and Immunology, University of Vigo, Spain | 24th March, 2015 |
| 34. | Replication and modulation of innate immunity by hepatotropic and modulation of innate immunity by hepatotropic RNA viruses | **Dr. C T Ranjith Kumar**<br>THSTI, Faridabad, Haryana | 25th March, 2015 |
| 35. | Of glia and macrophages | **Dr. Angela Giangrande**<br>IGBMC Strasbourg, France | 31th March, 2015 |





# CONFERENCE/SYMPOSIUM/ WORKSHOPS ORGANIZED AT NII

**Workshop on Plagiarism**

**Dr. Ramesh C. Gaur**, University Librarian, JNU delivered a lecture on **'What is Plagiarism and how to detect & avoid it ?'** and **Dr. Manorama Tripathi**, Deputy Librarian, JNU Central Library, New Delhi conducted **'Orientation Session on Turnitin'** at NII Auditorium on 17th July 2014.




**DBT-NCI Provocative Question Workshop on Cancer Biology**

The Department of Biotechnology (DBT) in association with the National Cancer Institute (NCI), USA co-sponsored a series of Provocative Questions workshops on Cancer Biology, in India. NII hosted the first workshops on 27th October, 2014 as part of an Indo-US workshop to boost research and discuss provocative questions about cancer.



*Participants at the DBT-NCI Provocative Question Workshop on Cancer Biology*

**Symposium on Infection and Immunoregulation**

Symposium on **"Infection and Immunoregulation"** was conducted on 27th -29th November, 2014 at the Institute to provide a platform for interaction amongst scientists of the Institute and Singapore Immunology Network (SIgN), to identify potential areas for collaboration.




**Indo Canadian Workshop NCR Bio-Cluster and Department of Immunology, University of Toronto, Canada**

A partnership workshop for exploration of collaborative activities was organized in collaboration with the Immunology Department, University of Toronto, Canada was held on 14th-15th January, 2015 at the Institute.



## International Congress

International Congress on **"Embryo Implantation and Pregnancy: Intricacies and Strategies for its Success"** was held at NII on 9th -11th March, 2015. The aim of this Congress was to act as catalyst by bringing various scientists and clinicians working in this area at a common platform and to have an assessment of the status and future needs for scientific investigations. The Congress covered both mechanistic and clinical aspects of the feto-maternal relationship.




## Science Setu

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. National Institute of Immunology launched a **'Science Setu Programme'** on 26th March, 2015. Ten University of Delhi Colleges; Daulat Ram College, Dyal Singh College, Gargi College, Hansraj College, Hindu College, Miranda House, Ramjas College, SGTB Khalsa College, Sri Venkateswara College and Zakir Hussain Delhi College have agreed to be partners in the Science Setu programme.

**Dr. K. VijayRaghvan**, FRS and Secretary, Department of Biotechnology was the Chief Guest and he delivered a talk on "***Why we should stop studying (and have fun learning)***". He emphasised that the only path to success was through and hard work and love for whatever one does.






**Professor Raghavendra Gadagkar,** IISc, Bengaluru and President INSA, was the Guest of Honour and delivered a talk on "***Science Education Indisciplined***". He urged teachers and students to break boundaries of every kind.

About 300 people including principals, teachers and students from the partner colleges as well as scientists participated in the function.

# VISITORS TO THE INSTITUTE

1. **Prof. Kazuhiko Yamada**, Professor and Director, Centre for Advanced Biomedical Science and Swine Research, Kagoshima University, Japan visited the Institute on 5th September, 2014.

2. **Shri Y. S. Chowdary**, Hon'ble Minister of State for Ministry Science and Technology & Ministry of Earth Sciences, visited the Institute on 24th December, 2014.



*Hon'ble Minister Shri Y. S. Chowdary interacting with Research Scholar during his visit.*

3. Representatives of Université de Montréal's Institute for Research in Immunology and Cancer, Canada headed by **Dr. Sandra Jacquesona** visited the Institute on 18th November, 2014.

4. Delegation of CNRS (*Centre National de la Recherche Scientifique*) headed by **Dr. Catherine Jesus**, Director of the Institute of Biological Sciences (INSB), France visited the Institute on 6th February, 2015.

# NOTABLE ACTIVITIES

## ACADEMIC COURSES, TRAINING PROGRAMMES AND INTERACTION WITH OTHER ACADEMIC INSTITUTES

The Institute imparts long-term residential training leading to Ph.D. Degree of the Jawaharlal Nehru University, New Delhi. Every year 35-40 scholars are admitted to this Programme on competitive basis after an examination and interviews amongst a large number of applicants from all over the country.

The Ph.D. Programme of the Institute was launched in the academic year 1986-87. Since then, the Institute has admitted 619 students in 29 batches. Till now, 338 students have been awarded the PhD degree including 25 that have obtained the degree in academic year 2014-15. Many others are at various stages on their research work for the degree.

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

## IMPLEMENTATION OF OFFICIAL LANGUAGE POLICY

The Official Language policy of the Government of India is followed in 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 first to 14th September 2014. During this period, various Hindi competitions such as Hindi Sulekh (Hindi Writing), Hindi Nibandh (Hindi Essay), Hindi Shrutlek (Hindi dictation), Hindi Vaad-vivad (Hindi Debate), Hindi Samanya Gyaan (General Knowledge Competition) and Hindi Kavita Pathan (Hindi Poetry recitation) were organized in the Institute, wherein a large numbers of faculty members, staff members and students had participated. Hindi Diwas (Hindi Day) was celebrated on 15th September 2014 on the culmination of Hindi Pakhwara.

Hindi Workshop on "Use of I.T Applications and Unicode at Computer in Hindi" was organized for the Staff members to remove their hesitation of carrying out Official work in Hindi on Computer. As NII is a scientific Institution, the member of Commission for Scientific and Technical Terminology, New Delhi also organized a lecture on "Scientific and Technical writings in the Hindi language format" on 5th August 2014.

The Committee of Parliament on Official Language carried out a Rajbhasa Inspection of the Institute on 12th January 2015 and appreciated efforts made by the NII for implementation of Official Language policy in its Official works.

## INDEPENDENCE DAY CELEBRATION

Independence Day was celebrated in the Institute on 15th August 2014. The event was marked by Independence Day Message from the Director, followed by the National Anthem by the students and children of the staff of the Institute.



*NII Staff celebrating Independence Day at the campus.*

## FAREWELL TO Ph.D STUDENTS

Farewell function of the 2009 batch of PhD Students with Staff Scientists, which was marked by planting of tree by the students at the Institute.



*Group Photo of Ph.D 2009 Batch with Director and Staff.*

## ANTI-TERRORISM DAY, SADHBHAVNA DIWAS AND COMMUNAL HARMONY WEEK

Anti-Terrorism Day was observed by all employees of the Institute on 21st May 2014 by taking anti-terrorism/violence pledge stating: 'We, the people of India, having abiding faith in our country's tradition of non-violence and tolerance, hereby solemnly affirm to oppose with our strength, all forms of terrorism and violence. We pledge to uphold and promote peace, social harmony and understanding among all fellow human beings and fight the forces of disruption threatening human lives and values'.

With the theme to promote national integration and communal harmony among people of all religions, languages and regions, 'Sadhbhavna Diwas' was observed in the Institute on the birth anniversary of late Shri Rajiv Gandhi on 20 Aug 2014 by taking pledge by each staff that 'I take this solemn pledge that I will work for the emotional oneness and harmony of all the people of India regardless of caste, region, religion of language. I further pledge that I shall resolve all differences among us through dialogue and constitutional means without resorting to violence'. To promote the idea further a fortnight from 20 Aug to 3 Sep 2014 was observed as Communal Harmony Week. A Communal Harmony Campaign and Fund Raising Week was observed in the in the Institute from 19 to 25 Nov 2014 and Flag Day on 25 Nov 2014, when the funds for rehabilitation of child victims of communal violence were collected and sent to the National Foundation for Communal Harmony.

## 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.

## REPRESENTATION OF PERSONS WITH DISABILITIES

The Institute follows reservation orders for Persons with Disabilities as per Govt. of India directives issued from time to time to ensure representation of persons with disabilities as per the prescribed percentage. During the reporting period, the Institute had re-advertised one Group 'A' backlog reserved vacancy (for Staff Scientist-II) under Special Recruitment Drive, in National dailies including Employment News. The vacancy was also circulated among Special Employment Exchanges for Physically Handicapped for seeking nomination. Selection Committee was constituted and the outcome would be informed in the next Annual Report.


Applications of Mass Spectrometry for Proteomics
THERMO SPARES,
CD's. ION VOLUME.

# INFRASTRUCTURE

## RESEARCH FACILITIES

### *EQUIPMENT*

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

### *BSL-III FACILITY*

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

The propagation of all defined strains is done in a three-tier system i.e., the Foundation Stock (FS), Pedigreed Expansion Stock (PES) and Production Stock (PS). The selection of sibling pairs at these three levels is done in such a way that all the descendants of each strain can be traced to a common ancestral breeding pair within seven generations of inbreeding. Genetically modified mouse strains are bred either by 1.Homozygous mutant (-/-) x homozygous mutant (-/-) 2. Heterozygous mutant (-/+) x homozygous mutant (-/-) 3.Heterozygous mutant (-/+) x heterozygous mutant (-/+)

Defined breeding protocols and careful management and husbandry procedures are followed to ensure the purity of each strain of mice. To maximize genetic purity and uniformity of mice, inbred strains are propagated in such a manner that minimizes the genetic drift and the number of generations, which separate breeding stock in production colonies from their ancestors in foundation colonies. A random sample of few retired breeders of Foundation, Expansion and Production stock are monitored with the help of genetic and biochemical markers to ensure their genetic purity. The facility also gets support from various principal investigators in the genotyping of transgenic and knockout mice strains to confirm the genetic purity based

on presence or absence of the selected gene of interest.

Health monitoring procedures includes microbiological examination of feed, water, bedding material, stool samples and animal organs from moribund animals. Health monitoring program includes screening of Mouse Hepatitis virus, Mouse Parvovirus, Mouse Norovirus, Pnemonia virus of Mice, Mycoplasma and Sendai virus using Elisa and PCR. Bacterial pathogens such as *Pseudomonas aeroginosa, Streptobacillus moniliformis, Bordetella, Bronchiseptica, Citrobacter rodentitium, Pasteurella pneumotropica, Staphylococci* and *E.Coli* are screened using culture, biochemical and PCR methods. Faecal samples are randomly selected for the presence of endoparasites by sedimentation method for the presence of syphacia and aspicularis species. In addition, periodic FACS analyses are done on immunodeficient mice for their leakiness.

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

***PRIMATE RESEARCH CENTRE***

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

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

Protein rich pellets containing appropriate content of fat, carbohydrate and vitamin are provided to monkeys ad libitum. In addition to this, bread, germinated gram, vegetables and/or fruits are also given daily. For change of taste, occasional feast like bread with sauce or jaggery coated groundnuts are given. Breast

feeding mothers and pregnant females are given calcium and vitamin supplements on bread. Care is taken to provide excess feed to such females. Drinking water is provided to the animals by pipelines behind monkey cages, which are connected to flexible protective hose-pipe at the top of each cage. Steel nozzles with Teflon interior are fitted at the tip of these hosepipes for the continuous access to drinking water. To make the staff aware of or to remind preventive measures for health safety, occasional meetings are held with the staff and they are mentored very often. The attendants are provided with overall, jacket, pyjama and footwears for use during animal handling and cleaning. Use of gloves and mask is mandatory during work. Booster of TT is given once every year. The staff also receives boosters of anti rabies vaccine when required. TB test and chest x-ray of staff and the security personnel are performed occasionally. As a preventive measure, persons having injury are given non-animal work. Every precaution is taken to prevent cross species infection; monkey to human and vice versa. High-grade sanitary norms are followed for cleaning in the monkey rooms and area surrounding the building by using disinfectants and insecticides. To prevent colonization of microbes the sewer channels and tiles of room are routinely cleaned.

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

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



Administrative and Supporting  Staff with Senior Manager Mr. N. S. Padmanabhan

# SUPPORTING UNITS

## SUPPORTING UNITS

### Establishment, Personnel and General Administration Services

The Division has been providing effective administrative support in terms of manpower employment, liaison, secretarial assistance and infrastructure to meet its goal of coordination of human and material resources. The activities include service matters, policy implementation, preparation and submission of periodic reports to nodal Ministry, postal-mail, foreign visits of scientists for training, conferences, exchange visit etc. The Institute also conducts periodical trainings for its Administrative & Technical Cadre on subjects of relevance to them. The activities are performed with modern gadgets like fax machines, computers, e-mails etc. In addition to the routine jobs, the staffs of Administration has also been involved in other activities like maintenance of building, residential complex, swimming pool, guest house, organization of various seminars, workshops, training courses, lectures etc.

### Financial and Accounting Services

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

### Stores and Purchase Department

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

**Engineering, Maintenance and Instrumentation Services**

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

i) Installation of sewage treatment plant. ii) Creation of storage space in existing Incinerator Room. iii) Up gradation of Cold rooms. iv) Setting up of New Laboratories & Offices. v) Creation of space for TEM facility. vi) Supply, retrofitting & commissioning of 1250 Amp ACB along with hardware's, vii) Supply of material, service & overhauling of cooling towers of New experimental Animal Facility. viii) Fabrication of wooden racks for cell biology lab-I. ix) Fabrication of cabin at mezzanine floor of new admin Area. x) Reconstruction of the officer's cabin of finance branch. xi) Fabrication of laboratory working table, side racks, PVC flooring for various labs. xii) Providing & fixing of aluminum partition, doors & fabrication of office table/racks at various locations.

The department is currently working on the following projects:

i) Installation of rain harvesting system. ii) Construction of boundary wall at Sector V Dwarka. iii) Fabrication of enclose at PRC. iv) Setting up of New Laboratories v) Creation of warm culture Room. vi) Supply, Installation, Testing & commissioning of 1250 KVA D.G. Set. vii) Servicing & repairing of various DG Sets to maintain back up supply in healthy condition. viii) Installation of Solar System for street light. ix) Installation of solar system for sharing regular electrical load.

**Library and Documentation Services**

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

Library has recently set up 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 enhanced 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 desktop 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 its patron. Library involved in the process of compilation of Annual Report of the Institute and purchasing process of print and online Journals, books and publications.

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

popularizing the official language amongst staff of the Institute.

**Academic and Training Services**

The activities of the Academic & Training Department are grouped under three major groups viz. Students Affairs, Outside Training, and In-House training. The unit has been involved in Ph.D. admissions, Pre-PhD registration courses, Doctoral Committee meetings, Academic Committee meetings, Fellowship of scholars, Indian Institute of Science. Department of Bio-technology- Post Doctoral Fellowship (DBT-PDF), Indian Council of Medical Research- Research Associates (ICMR- RAs), Wellcome Trust Fellow and Department of Science and Technology Women Scientist (DST-WOS). The unit has 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 organizations 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 Chief Vigilance Commissioner (CVC).The CVO and the support staffs performs vigilance functions as adjunct duties in addition to their primary responsibilities. The Cell followed various instructions issued by the CVC from time to time to ensure effective implementation of the measures outlined in the instructions, and strengthening vigilance and anti-corruption work. Emphasis is laid on preventive vigilance since such efforts, if properly conceived and executed aids in plugging weak and vulnerable areas. The Institute reviews existing procedures to identify corruption prone areas, making policies more transparent to avoid ambiguity and streamlining procedures to achieve a corruption-free environment. Plans for rotation of staff employed in areas potentially prone to corruption have been implemented. 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. The composition of the purchase committees are reviewed and altered after a reasonable period. The Institute has thus far been able to maintain a clean slate in corruption matters. 'Vigilance Awareness Week' was observed in the Institute from October 27 to November 01, 2014 during which a pledge to fight against corruption was taken on October 27, 2014. The Cell has been rendering periodical reports and returns on vigilance activities to the administrative machinery and CVC.

**Computer Centre**

Computer Centre has been providing all Information Technology related support to NII, which involve managing switches and Wi-Fi controllers in a 600 node LAN, system administration of multiple LINUX based E-mail and Web servers, backup services for mail/web servers, managing UTM devices for network security and integrating internet bandwidth from multiple ISPs. Computer center staff facilitates day to day trouble shooting, maintenance and anti-virus support of about 700 PCs and other peripheral devices. In addition Computer center also provides specialized services like management of HPC clusters, managing floating licenses for access to Bioinformatics softwares over LAN and IT support for developing in house software for Pay Roll and maintenance of employee database.



# ORGANIZATION

**NII SOCIETY**

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

Prof. K. VijayRaghavan
Chairman, G.B. NII & Secretary
Department of Biotechnology
New Delhi

Dr. Jagdish Prasad
Director General Health Services
Ministry of Health & Family
Welfare
Nirman Bhawan
New Delhi

Prof. M.C. Misra
Director, AIIMS
New Delhi

Dr. V. M. Katoch
Secretary, DHS & Director General
ICMR, V. Ramalingaswami
Bhawan
New Delhi

Dr. S. Ayyappan
Director General, ICAR
New Delhi

Prof. Ved Prakash
Chairman, UGC
New Delhi

Prof. Sudhir K. Sopory
Vice Chancellor, JNU
New Delhi

Prof. V. Nagaraja
Professor
Department of Microbiology &
Cell Biology, IISc
Bangalore

Dr. Anuradha Lohia
Vice Chancellor
Presidency University
Kolkata

Ms. Deepanwita Chattopadhyay
Managing Director & CEO
IKP Knowledge Park
Secunderabad

Mr. J. B. Mohapatra
Joint Secretary & Financial Advisor
Ministry of Science & Technology
Department of Biotechnology
New Delhi

Dr. Chandrima Shaha
Director,
National Institute of Immunology
Aruna Asaf Ali Marg,
New Delhi

## GOVERNING BODY

Prof. K. VijayRaghavan
(Chairman)
Secretary
Department of Biotechnology
New Delhi

Dr. Jagdish Prasad
Director General Health Services
Ministry of Health & Family
Welfare,
New Delhi

Prof. M.C. Misra
Director, AIIMS
New Delhi

Dr. V. M. Katoch
Secretary, DHS & Director General
ICMR
New Delhi

Prof. Sudhir K. Sopory
Vice Chancellor, JNU
New Delhi

Dr. Anuradha Lohia
Vice Chancellor
Presidency University
Kolkata

Dr. S. Ayyappan
Director General
ICAR
New Delhi

Mr. J. B. Mohapatra
Joint Secretary & Financial Advisor
Ministry of Science & Technology
Department of Biotechnology
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Ms. Deepanwita Chattopadhyay
Managing Director & CEO
IKP Knowledge Park
Secunderabad

Prof. Ved Prakash
Chairman
UGC
New Delhi

Prof. V. Nagaraja
Professor
Department of Microbiology & Cell
Biology, IISc
Bangalore

Dr. Chandrima Shaha
Director,
National Institute of Immunology
New Delhi

## SCIENTIFIC ADVISORY COMMITTEE

Prof. M. Vijayan
(Chairman)
INSA Albert Einstein Professor
Molecular Biophysics Unit, IISc
Bangalore

Dr. Ranjan Sen
Chief, Laboratory of Molecular
Biology & Immunology
Biomedical Research Centre
Baltimore, MD
USA

Dr. Lalita Ramakrishnan
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Adjunct Professor, Immunology
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University of Washington
Seattle WA
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President
NII Society & INSA Senior Scientist,
Senior Science Innovation Advisor
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Director
Rajiv Gandhi Centre for
Biotechnology
Thycaud, Jagathy
Thiruvananthapuram

Prof. Subrata Sinha
Director
National Brain Research Centre
Manesar
Haryana

Prof. Ashok Venkitaraman
Ursula Zoellner Professor,
Director, MRC Cancer Cell Unit
University of Cambridge
UK

Dr. Uttam Surana
Institute of Molecular & Cell Biology
Proteos
Singapore

**RESEARCH AREA PANEL**

Dr. Anand K. Bachhawat
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Indian Institute of Science Education & Research (IISER) Mohali
Punjab

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IISc
Bangalore

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Prof. D. N. Rao
Professor
Department of Biochemistry
IISc, Bangalore

Dr. Saumitra Das
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Dr. Apurva Sarin
Dean, inStem & Head (Academics)
National Centre for Biological Sciences
Bangalore

Dr. Shubhada Chiplunkar
Director,
Advanced Centre for Treatment Research & Education in Cancer
Tata Memorial Centre
Navi Mumbai

Dr. R. Nagaraj
Scientist
Centre for Cellular & Molecular Biology
Hyderabad

Dr. Rajan Sankaranarayanan
Group Leader
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Professor of Genetics and Director (Coordinator), NCAHG
School of Life Sciences, JNU
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Director,
Dr. B.R. Ambedkar Center for Biomedical Research,
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(North Campus) Delhi

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Kalyani

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Department of Gastrointestinal Sciences
Christian Medical College
Vellore

**FINANCE COMMITTEE**

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Secretary
Department of Biotechnology
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Dr. Neeta Singh
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Mr. J. B. Mohapatra
Joint Secretary & Financial Advisor
Ministry of Science & Technology
Department of Biotechnology
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Dr. Chandrima Shaha
Director,
National Institute of Immunology
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Mr. N.S. Padmanabhan
(Non-member Secretary)
Senior Manager,
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## BUILDING COMMITTEE

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(Former President of the Council of Architecture)
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Registrar,
Jawaharlal Nehru University
New Delhi

Prof. M.C. Misra
Director, AIIMS
New Delhi

Dr. S. V. Gangal
(Former Diector, IGIB, Delhi)
Mumbai

Mr. Krishen Khanna
Artist
Panchsheel Park
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Mr. J.B. Mohapatra
Joint Secretary & Financial Advisor
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Department of Biotechnology
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Mr. I. K. Puri
Architect, Panchsheel Park
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Dr. Chandrima Shaha
Director,
National Institute of Immunology
New Delhi

Mr. N.S. Padmanabhan
(Non- Member Secretary)
Senior Manager,
National Institute of Immunology
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## ACADEMIC COMMITTEE

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Director,
National Institute of Immunology
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Professor
Special Centre for Molecular Medicine
JNU, New Delhi

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Prof. Anil K. Tyagi
Vice Chancellor
Guru Gobind Singh Indraprastha University, New Delhi

Prof. K. Natarajan
Director,
Dr. B.R. Ambedkar Center for Biomedical Research
University of Delhi,
Delhi

Prof. Bhupinder Zutshi
(Special Invitee)
Officiating Controller of Examination
JNU, New Delhi

Dr. Vineeta Bal
Staff Scientist, NII
New Delhi

Dr. Rahul Pal
Staff Scientist,NII
New Delhi

## INSTITUTIONAL BIO-SAFETY COMMITTEE

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New Delhi

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Director, NBRC
Gurgaon

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ICGEB
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New Delhi

Dr. Satyajit Rath
Staff Scientist, NII
New Delhi

Dr. Sandeep Saxena
Staff Scientist, NII
New Delhi

## INSTITUTIONAL ANIMAL ETHICS COMMITTEE

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Staff Scientist, NII
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Dr. Ayub Qadri
(Convenor)
Staff Scientist, NII
New Delhi

Dr. Vijay Pal Singh
(CPCSEA Link Nominee)
New Delhi

Dr. J.P. Govekar ,
(CPCSEA Nominated Member)
New Delhi

Dr. B. B. Batra,
(CPCSEA Nominated memebr)
New Delhi

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University College of Medical Sciences
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Staff Scientist, NII
New Delhi

Dr. Madhulika Srivastava
Staff Scientist, NII
New Delhi

Dr. P. Nagarajan
Staff Scientist, NII
New Delhi

**INSTITUTIONAL HUMAN ETHICS COMMITTEE**

Prof. Subrata Sinha
(Chairman)
Director, NBRC
Manesar, Gurgaon

Dr. Chitra Sarkar
(Alternate Chairperson)
AIIMS, New Delhi

Dr. Sandeep Mathur
AIIMS, New Delhi

Dr. Goutam Bhattacharya
K&S Partners,
Intellectual Property Attorneys
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Dr. Shinjini Bhatnagar
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THSTI,
Faridabad

Maj. Gen. B. S. Dhillon, VSM,
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201 Rose Apartments,
Gurgaon

Mr. Rajinder Raina
NIPGR,
New Delhi

Dr. Akhil C. Banerjea
(Secretary)
Staff Scientist, NII
New Delhi

## STAFF OF THE INSTITUTE

(As on 31.03.2015)

***CORE & INFRASTRUCTURE SCIENTISTS***

Dr. Chandrima Shaha, Ph.D, Director
Dr. Dinakar M. Salunke, Ph.D, Deputy Director*
Dr. Janendra K. Batra, Ph.D, Deputy Director
Dr. Satish K. Gupta Ph.D, Deputy Director
Dr. Akhil C. Banerjea, Ph.D, Staff Scientist-VII
Dr. Amitabha Mukhopadhyay, Ph.D, Staff Scientist -VII
Dr. Amulya K. Panda, Ph.D, Staff Scientist-VII
Dr. Anil K. Suri, Ph.D, Staff Scientist-VII
Dr. Anna George, Ph.D, Staff Scientist-VII
Dr. Lalit C. Garg, Ph.D, Staff Scientist-VII
Dr. Rajendra P. Roy, Ph.D, Staff Scientist-VII
Dr. Rajesh S. Gokhale, Ph.D, Staff Scientist-VII*
Dr. Rajni Rani, Ph.D, Staff Scientist-VII
Dr. Satyajit Rath, Ph.D, Staff Scientist-VII
Dr. Sher Ali, Ph.D, Staff Scientist-VII
Dr. Subeer S. Majumdar, Ph.D, Staff Scientist- VII
Dr. Vineeta Bal, Ph.D, Staff Scientist-VII
Dr. Debasisa Mohanty, Ph.D, Staff Scientist-VI
Dr. Kanwaljeet Kaur, Ph.D, Staff Scientist-VI
Dr. Madhulika Srivastava, Ph.D, Staff Scientist- VI
Dr. Mohd. Ayub Qadri, Ph.D, Staff Scientist-VI
Dr. Pushkar Sharma, Ph.D, Staff Scientist-VI
Dr. Pramod K. Upadhyay, Ph.D, Staff Scientist-VI
Dr. Rahul Pal, Ph.D, Staff Scientist-VI
Dr. Sagar Sengupta, Ph.D, Staff Scientist-VI
Dr. Sangeeta Bhaskar, Ph.D, Staff Scientist-VI
Dr. Vinay K. Nandicoori, Ph.D, Staff Scientist-VI
Dr. Agam P. Singh, Ph.D, Staff Scientist-V
Dr. Apurba Kumar Sau, Ph.D, Staff Scientist-V
Dr. Arnab Mukhopadhyay, Ph.D, Staff Scientist-V
Dr. Bichitra K. Biswal, Ph.D, Staff Scientist-V
Dr. Devinder Sehgal, Ph.D, Staff Scientist-V
Dr. Monica Sundd, Ph.D, Staff Scientist-V
Dr. Sandeep Saxena, Ph.D, Staff Scientist-V
Dr. Sanjeev Das, Ph.D, Staff Scientist-V
Dr. S. Gopalan Sampathkumar, Ph.D, Staff Scientist-V
Dr. Prafullakumar B. Tailor, Ph.D, Staff Scientist-IV
Dr. Sarika Gupta, Ph.D, Staff Scientist-IV
Dr. Soumen Basak, Ph.D, Staff Scientist-IV
Dr. Vidya Raghunathan, Ph.D, Staff Scientist-IV
Dr. Parmeshwari Sahai, Ph.D, Staff Scientist-III
Dr. Anil Kumar, Ph.D, Staff Scientist-II
Dr. P. Nagarajan, MS (Veterinary), Staff Scientist-II

** On Lien/ Deputation*

**OTHER SCIENTIFIC STAFF**
(as on 31.03.2015)

***Project Scientists***
Dr. Arumaugam Madumallar
Dr. Nirmala Jagadish
Dr. Viji Vijajan

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# A page from history




Prof. R. N. Dogra inaugurating the Cafeteria Block of NII on the 29th October, 1991, while Prof. G. P. Talwar, Founder Director looks on.

# NII Collaborations




| | | |
|---|---|---|
| 1 | **Aligarh** | AMU |
| 2 | **Allahabad** | University of Allahabad |
| 3 | **Bangalore** | IISc, IOB, Christian Blind Mission, IBAB, St John's Medical College |
| 4 | **Bhubaneswar** | RMRC, ILS, KIIT University |
| 5 | **Chennai** | Cancer Institute (WIA), |
| 6 | **Delhi/NCR** | AIIMS, University of Delhi, JNU, THSTI ,ILBS ,INMAS ,St. Stephen's Hospital , Jamia Hamdard,CSIR-IGIB, JMI, TRF,I CARE Eye Hospital, Safdarjung Hospital ,VMM College, RCB ,VIMHANS Hospital, National Institute of Pathology, RML Hospital ,IOP, UCMS/GTB Hospital ,IIT Delhi |
| 7 | **Hyderabad** | CCMB, CDFD, BITS |
| 8 | **Jaipur** | MG Medical College and Hospital, University of Rajasthan |
| 9 | **Kanpur** | IIT |
| 10 | **Kolkata** | IICB |
| 11 | **Lucknow** | IITR, CDRI, SGPGI, |
| 12 | **Mumbai** | NIRRH, ACTREC, NIRRH, ACTREC |
| 13 | **Pune** | CSIR-NCL , NCCS |
| 14 | **Sagar** | DHG Vishwavidyalaya |
| 15 | **Shantiniketan** | Visva-Bharati |
| 16 | **Tezpur** | Tezpur University |
| 17 | **Thiruvantapuram** | HLL Lifecare Ltd |
| 18 | **Vadodara** | Maharaja Sayajirao University of Baroda |
| 19 | **Varanasi** | BHU |

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4. Ohio State University, Ohio, USA
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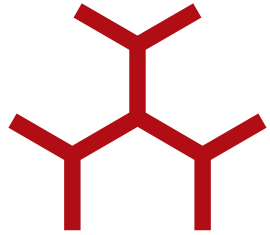

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