Research Work Overview.

I have established my laboratory 20 years ago at the National Institute of Immunology, New Delhi. I moved to the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, three years ago as the Director. I moved my laboratory from NII to CCMB. Tuberculosis has been a long-standing problem in our country and due to the emergence of drug-resistant strains, the search for new drug targets continues. Its complex physiology and ability to survive in hostile environments, coupled with the serious rise in multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis cases, has necessitated the renewal of efforts to understand the molecular basis of its pathogenesis. To effectively treat tuberculosis, it is imperative to find newer targets, which are important for *in-vivo* bacterial survival and persistence.

My laboratory at the Centre for Cellular and Molecular Biology, Hyderabad & previously at the National Institute of Immunology, New Delhi, has been working on "**delineating the molecular mechanism involved in modulating the survival of *Mycobacterium tuberculosis (Mtb)***" for the past 20 years. The work involves investigating various multiple cellular processes such as serine/threonine kinase-mediated signaling, transcription initiation & elongation and its regulation, cell division in mycobacteria, enzymes of cell wall synthesis, the evolution of drug resistance, and examining host mechanisms exploited by the pathogen.

In addition to our research on *Mtb*, we also worked on deciphering the role of phosphorylation in modulating the function of nucleoporin Tpr. We stopped on working on this project in 2014 after publishing three manuscripts and when student working on the project graduated. (***Mol. Cell Biol.*** (2008) 22, 6954-6966; ***Plos One*** (2012) **7**, e29921; ***J of Cell Science*** (2014) **127**, 3505).

Role of phosphorylation events in modulating the functions of nucleoporins.

- A major constituent of the nuclear basket region of the nuclear pore complex (NPC), nucleoporin Tpr, regulates multiple important processes. We have established that Tpr is phosphorylated in both a MAP-kinase-dependent and independent manner and that Tpr acts as both a substrate and a scaffold for ERK2 (***Mol. Cell Biol.*** (2008) 22, 6954-6966).
- Investigations by our group showed that localization of nucleoporin Tpr is essential for Tpr-mediated regulation of the export of unspliced RNA (***Plos One*** (2012) **7**, e29921).
- We reported the identification of S2059 and S2094 as the major novel ERK-independent phosphorylation sites *in vivo* in the Tpr protein. We found that abrogation of S2059 phosphorylation abolishes the interaction of Tpr with Mad1, thus compromising the localization of both Mad1 and Mad2 proteins, resulting in cell cycle defects (***J of Cell Science*** (2014) **127**, 3505).

Deciphering the role of cell signaling in Mtb biology

Protein Kinases and phosphatases in Mtb transduce extracellular stimuli to a cellular response, ensuing the pathogen's growth, persistence, and pathogenesis. We are interested in discovering signaling networks in *Mtb* and delineating how the STPKs regulate multiple cellular processes, such as cell division, cell wall synthesis, secretion, transcription, and survival in the host.

Deciphering STPK mediated regulation of cellular processes in Mtb.

Deciphering kinase-mediated signaling networks in Mtb

Protein phosphorylation has become a preeminent circuitry regulating many physiological processes in the bacterial kingdom. There are 11 eukaryotic-like STPKs in *Mtb*, and we have worked towards analyzing the functional roles of the phosphorylation events mediated by these kinases.

- Protein kinases A and B, encoded by *pknA* and *pknB,* respectively, are part of the operon carrying cistrons coding for protein phosphatase *pstP, rodA* (involved in cell shape control), and *pbpA* (involved in peptidoglycan synthesis). Work from the lab elucidated the roles of RodA and PbpA in regulating *in vitro* growth and *in vivo* survival of pathogenic mycobacteria (***J. Biol. Chem***. (2018) **293**, 6497).
- Using the mouse infection model, we observed that PknA is essential for the survival of the pathogen in the host. Even though PknA and PknB are expressed as part of the same operon, they appear to be regulating cellular processes through divergent signaling pathways (***J Biol Chem***. (2015) **290,** 9626-9645).
- While there are 11 serine/threonine protein kinases in *Mtb*, only one serine/threonine phosphatase, PstP, has been identified. PstP depletion results in elongated multiseptate cells, suggesting a role for PstP in regulating cell division events. PstP plays an important role in establishing and maintaining infection, possibly via the modulation of cell division events. (***J Biol Chem***. (2016) **291,** *24215-30).*
- A particular class of receptor-type serine-threonine kinase called PASTA (**P**enicillin-binding proteins **A**nd **S**erine **T**hreonine **A**ssociated) kinase is widespread across Gram-positive firmicutes and actinomycetes. We found that stringent regulation of *Mtb* of the PASTA kinase PknB ($PknB_{Mtb}$) to be necessary for cell survival. While the presence of the carboxy-terminal PASTA domain is dispensable in the avirulent *M. smegmatis*, all four PASTA domains are essential in *Mtb (**J. Biol. Chem***. (2014) **289**, 13858-75).

- With the help of *in silico* molecular simulations, we identified potential ligand-binding residues in the linker regions between PASTA3 and 4 domains. The extracytoplasmic domain interacts with mDAP-containing LipidII, and this is abolished upon mutation of the ligand-interacting residues.

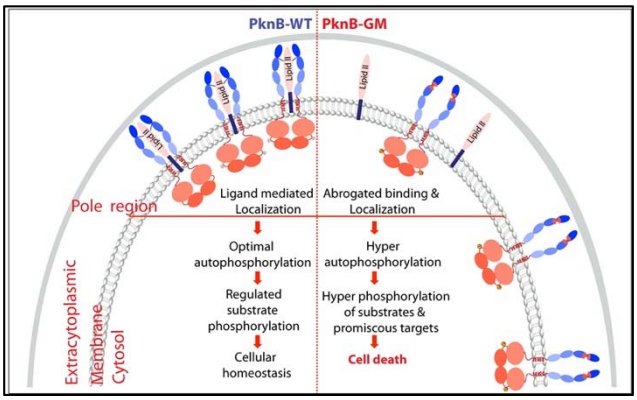

Figure 1. Model depicting PknB regulation wherein LipidII interacts with a specific region of PASTA 3-4 linker region of PknB and defines its localization to polar/septal niches and regulates the activity to optimal levels and hence maintains cellular homeostasis and cell survival.

  Contrary to the prevailing hypothesis, abrogation of ligand-binding is linked to activation loop hyperphosphorylation and indiscriminate hyperphosphorylation of PknB substrates as well as other proteins, ultimately causing loss of homeostasis and cell death. (*Nature Communications* (2019) **10, 1231**).
- Protein kinase G (PknG), a thioredoxin-fold–containing eukaryotic-like serine/threonine protein kinase, is a virulence factor in *M. tuberculosis*, required for inhibition of phagolysosomal fusion. We showed for the first time that the expression of PknG in non-pathogenic mycobacteria allows the continued existence of these bacteria in host tissues (*J Biol. Chem.* (2009) 284, 27467-27479).
- We unraveled novel functional facets of PknG during latency-like conditions. We proposed that PknG probably acts as a modulator of latency-associated signals (*J. Biol. Chem*. (2017) **292,** 16093-16108).
- Recently, we investigated the therapeutic potential of targeting PknG against latent mycobacterium. In the Cornell mouse model of latent TB, the deletion of *pknG* drastically attenuated *Mtb's* ability to resuscitate post antibiotics treatment. Collectively, the results suggest PknG may be a promising drug target for adjunct therapy to shorten the treatment duration and lower disease relapse (*Antimicrob. Agents Chemother* (2021).
- We were the first to characterize PknK and show that PknK modulates the activation of transcription from the promoter of mycobacterial monooxygenase operon, through phosphorylation of the transcriptional regulator VirS (*J. Biol. Chem.* (2009) 284, 11090-11099).
- We showed that deletion of *pknL* causes an increase in the MIC of isoniazid. Collectively our data suggest that PknL plays a role in subverting redox stress and aids in better survival *ex vivo* and *in vivo* (*Tuberculosis (2021)*).

Substrates of protein kinases.

GlmU of Mtb is a promising target for therapeutic intervention.

- GlmU is an enzyme involved in the synthesis of UDP-GlcNAc, a key metabolite essential for the synthesis of peptidoglycan, disaccharide linker, arabinogalactan and mycothiol. We previously showed that GlmU is phosphorylated on T418 residue and its phosphorylation downregulates its acetyltransferase activity (*J. Mol. Biol* (2009) 20, 451-64).
- In collaboration with Dr. Balaji Prakash, we determined the structure of $GlmU_{Mtb}$ in complex with substrates bound at the acetyltransferase active site, and uncovered unique features in the carboxy terminus of $GlmU_{Mtb}$ (*J. Biol. Chem* (2012) 287, 39524-37).
- The absence of $GlmU_{Mtb}$ leads to extensive perturbation of bacterial morphology and substantial reduction in cell wall thickness under normoxic as well as hypoxic conditions. We have developed a novel anti-$GlmU_{Mtb}$ inhibitor (Oxa33), identified its binding site on $GlmU_{Mtb}$, and showed its specificity for $GlmU_{Mtb}$. The administration of Oxa33 to infected mice resulted in a significant decrease in the bacillary load. *Our study suggests that GlmU is a promising target for therapeutic intervention* (*Plos Pathogens* (2015) **11**(10), e1005235).
- We delineated the role of *ftsQ*, a terminal gene of the highly conserved division cell wall (dcw) operon, in growth, survival, and cell length maintenance in *Mtb* (*J. Biol. Chem* (2018) **293,** 12331).
- InhA, the primary target for the first line anti-tuberculosis drug isoniazid, is a key enzyme of the fatty-acid synthase II system involved in mycolic acid biosynthesis in *Mtb*. Our studies revealed that the phosphorylation of InhA by kinases modulates its biochemical activity, with phosphorylation resulting in decreased enzymatic activity (*J. Biol. Chem* (2010) **285,** 37860).
- In a study published in the *Journal of Infectious Disease (2021),* we suggested that Mtb's secreted PPiA interacts with the host integrin receptor, ensuing disease progression through the upregulation of host matrix metalloproteinases.

Comparative proteomic analyses of avirulent, virulent, and clinical strains of Mtb identified strain-specific patterns

We performed 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. Label-free quantification analysis of the data revealed 257 differentially expressed protein groups. This study revealed that strain-specific variations in protein expression patterns have a meaningful impact on the biology of the pathogen *(J. Biol. Chem. 291, 14257-73).*

Adjunctive host-directed therapy for the treatment of TB

A possible way to overcome the emergence of drug resistance is to combine antibiotic treatment that kills the bacteria with targeting host molecules that are necessary for the sustenance of infection, which is termed adjunct **h**ost-**d**irected **t**herapy (HDT). To identify new possibilities for HDT, we investigated the role of pathogen mediated double-stranded DNA damage and the role of host NAD+ dependent histone deacetylase Sirtuin 2.

Host sirtuin 2 as an immunotherapeutic target against tuberculosis

Mtb employs a plethora of mechanisms to hijack the host defence machinery for its successful survival, proliferation, and persistence. *Mtb* upregulates one of the key epigenetic modulators, NAD+ dependent histone deacetylase Sirtuin 2 (SIRT2), which, upon infection, translocates to the nucleus and deacetylates histone H3K18, thus modulating the host transcriptome, leading to enhanced macrophage activation. Pharmacological inhibition of SIRT2 restricts the intracellular growth of both drug-sensitive and resistant strains of *Mtb.* It enhances the efficacy of the front-line anti-TB drug Isoniazid in the murine infection model. SIRT2 inhibitor-treated mice display reduced bacillary load, decreased disease pathology, and increased *Mtb*-specific protective immune responses. This study links *Mtb* infection, epigenetics, and host immune response, which can be exploited to achieve therapeutic benefits ***(eLife*** (2020)***; 9:e55415).***

Mtb exploits host ATM kinase for survival advantage through SecA2 secretome

In response to the damage, the host activates an intricate and indispensable signaling cascade entitled "**D**NA **d**amage **r**esponse" (DDR) pathway, which detects and repairs the damaged lesions in DNA and regulates the activation of effectors that determine the cell's fate. Ataxia telangiectasia mutated (ATM), ATM- and Rad3-related protein (ATR), and DNA-dependent protein kinase catalytic subunit (DNA-PKcs) are three drivers of DDR Phosphorylation of H2AX at the serine 139 (γH2AX), at the site of damage is considered a marker for DNA damage. The effectors secreted by the SecA2 pathway are necessary and sufficient for inflicting genotoxic stress in the host. *Rv* inflicts DSBs to activate ATM, which in turn activates Akt, resulting in anti-apoptotic and pro-survival signals, which favors *Mtb* survival.

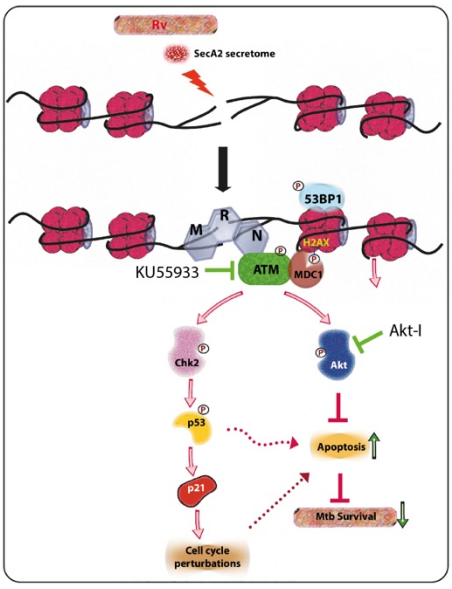

Figure 2: Rv induces genotoxicity and causes deleterious DSBs in the host genome through SecA2 secretome. Host cells, in response to the occurrence of DSBs, activate ATM kinase and are recruited at the site of damage by the sensor, MRN complex. pATM in a parallel pathway also activates Akt, inhibiting apoptosis and promoting cell survival. Activation of ATM and Akt and subsequent inhibition of apoptosis provides a survival advantage to Rv.

We have identified a novel survival mechanism utilized by *Mtb*, wherein the pathogen constantly challenges the host genome, activating pro-survival ATM-Akt signals (Figure 2). We propose using ATM inhibitors as an adjunct for HDT in treating tuberculosis (***eLife*** (2020) e51466).

Evolution of drug resistance in Mtb

The emergence of drug resistance in *Mtb* is alarming and demands in-depth knowledge for timely diagnosis. In a study published in ***Plos Pathogens (2021),*** we demonstrated that compromised repair in *Mtb* drives greater adaptability and provides a tool for facile identification of drug targets. Recently, we performed genome-wide association analysis using 2237 clinical strains of *Mtb* to identify novel genetic factors that evoke drug resistance. We showed that novel variant mutations in the DNA repair genes collectively compromise their functions and contribute to better survival under antibiotic/host stress conditions (***eLife*** (2023) e75860).

Redox homeostasis and regulation of cysteine synthesis.

Mycobacterium tuberculosis (*Mtb*) has evolved diverse cellular processes in response to the multiple stresses it encounters within the infected host. The analysis identified a single TF, Rv1332 (AosR), conserved across actinomycetes with a so-far uncharacterized function. Our study shows that the AosR-SigH pathway is critical for detoxifying host-derived oxidative and nitrosative radicals to enhance *Mtb* survival in the hostile intracellular environment (***EMBO J.*** (2021) 40: e106111). Using an integrated approach of microbial genetics, transcriptomics, metabolomics, animal experiments, chemical inhibition, and rescue studies, we investigated the biological role and therapeutic potency of non-canonical L-cysteine synthases, CysM and CysK2. Our work shows that CysM and CysK2 serve as unique, attractive targets for adjunct therapy to combat mycobacterial infection (***eLife (2024); RP91970***).

Regulation of transcription in Mtb

Transcription is a highly regulated process involving multiple players, such as RNA polymerase (which has four subunits), transcription initiation sigma factors, Nus elongation factors, proteins involved in termination, and more than 150 transcription factors. We are working on all the aspects associated with transcription. Our work showed that sigma factor A, the housekeeping sigma factor, is essential for growth at every stage of bacillary life and determined that the N-terminal and phosphorylation play a vital role in modulating functionality (*J. Biol. Chem.* 299(3):102933).

Administrative Responsibility

Three years ago, I took up Scientific administration as the Director of the Centre for Cellular and Molecular Biology, Hyderabad. I moved my lab from the National Institute of Immunology, New Delhi, to CCMB, Hyderabad. During this period, I published 15 manuscripts as a scientist, established a lab with seven students and seven project fellows, recruited 10 scientists, and sphere-headed the building of an auditorium and the refurbishment of the zebra facility. In addition, as the head of the Institute, I have led and been involved in major scientific programs such as the mRNA vaccine project, Rockefeller-funded surveillance project for SARS-CoV2, genome India project, and InTGS TB project, etc.

Summary

Mtb is an adaptable intracellular pathogen that exists in both dormant and active disease-causing states. Thus, work from the lab has increased our understanding of various aspects of *Mtb* cellular processes and given us significant insights into the role of cell signaling in mycobacteria and mammalian cells. *Our research on Mtb provided insights into different aspects of the pathogen's physiology and established strong candidates for developing newer drugs or for HDT.*

[Vinay Nandicoori]