Annexure-II

Details of research work carried out at CDFD for which the Sun Pharma Science Foundation Research Fellowship is claimed

The broad theme of our laboratory is to map the functional network of proteins controlling cellular homeostasis. We are currently focused on mapping the following networks

1. Phosphatase networks
2. Ubiquitin networks

While phosphatases regulate phospho-protein homeostasis, ubiquitin is essential for cellular protein homeostasis.

Phosphatases are a group of ubiquitously expressing enzymes, which are responsible for the removal of a phosphate group of various substrates in a cell. Despite being critical enzymes in the cell, a comprehensive analysis of their essential roles in various cellular processes and pathogenesis is not available. Phosphatases have been studied in isolation as enzymes but systematic studies to dissect the complex network of phosphatases and functional role of their interactions in different cellular processes were lacking. In our lab, we established a detailed interaction network of human phosphatases derived by a systematic proteomic analysis of 143 phosphatases using tandem affinity purification coupled with mass spectrometry. A total of 76773 interactions were obtained from 143 phosphatase purifications **(Kumar et al, J Proteome Research 2016)**. After filtering out the common contaminants using control GFP purification and eight different non-phosphatase purifications, we used Significance Analysis of Interactome (SAINT) algorithm to score protein-protein interactions. By using a SAINT score cut off of 0.9 and with spectral count above 3, we identified 6596 high confident interactions (HCIs) mediated by 2112 proteins (HCIPs) and 143 purified phosphatases. A comparison of our data with iRefIndex, a source of protein-protein interactions curated from various primary interaction databases, revealed that 6325 interactions by 1956 HCIPs were previously uncharacterized thus accounting for 95% of novel interactions in the list. Based on our phosphatase interactome, we already identified several novel cellular functions for different phosphatases. For instance, we discovered a novel functional role of PTEN in regulating endocytosis in phosphatase dependent and independent manner **(Shinde SR & Maddika S, Nature Communications 2016, Cell Reports 2017)**. We discovered a first example of a PPM family holoenzyme by PPM1G and B56δ to promote assembly of cell junctions **(Kumar et al., EMBO Reports 2019)**. On the other hand, we identified a novel role of tumor suppressor phosphatase in Kinetochore assembly **(Gangula NR & Maddika S, JBC 2013, JBC 2017)**.

Here, I present some of the key studies from this theme carried out in my lab for which the Sun Pharma Science Foundation Research Fellowship is claimed

1. SHP-1 mediated dephosphorylation of histone H2B acts as a molecular switch to facilitate transcription (Tathe et al., EMBO J. 2022).

Post-translational modifications of histones play an essential role during eukaryotic transcription. Among many modifications, reversible histone phosphorylation plays a critical role in providing a dynamic environment for recruitment of transcription factors, chromatin modifiers and effector proteins to cause productive transcription. While there has been lot of efforts in identifying kinases that promote histone phosphorylation, the role of phosphatases that maintain the phospho-homeostasis during these processes is relatively unexplored. On the other hand, histone ubiquitination H2B K120ub plays an important role during various steps of transcription cycle by acting as a signaling hub for various downstream biochemical processes. Whereas H2Bub exhibits cross talk with other histone modifications such as H3 lysine 4 (H3K4) and lysine 79 (H3K79) methylation, no cross talk exists between H2Bub and phosphorylation so far.

In this study, we discovered a new histone modification - H2B Y121 phosphorylation - that exhibits a functional crosstalk with H2B ubiquitination during transcription. Further, by analysing our interactome data, we identified SHP1 as a phosphatase that regulates this modification and promotes transcription. SHP-1 is a non-receptor tyrosine phosphatase that predominantly exist in cytoplasm of hematopoietic cells. We have shown that SHP-1 localizes to nucleus in various epithelial cells where it interacts with Paf1 complex and histone H2B. By using various biochemical assays, we demonstrated that nuclear SHP-1 dephosphorylates H2B at Y121 residue. We found that presence of phosphate group at Y121 inhibits the addition of ubiquitin to the adjacent K120 residue of H2B and therefore SHP-1 relieves this inhibition by dephosphorylating Y121 site. We clearly demonstrated that the cross talk between Y121 dephosphorylation and H2B ubiquitination is essential for productive transcription as SHP-1 loss led to defects in Pol II transition from initiation to elongation. Functionally, we have shown that SHP-1 dependent H2B dephosphorylation maintains basal autophagic flux in cells through efficient transcription of autophagy and lysosomal genes. Collectively, our study reveals an important modification of histone H2B regulated by SHP-1 that has an essential role during eukaryotic transcription (Summary in Figure 1) **(Tathe et al., EMBO J. 2022).**

2. EYA proteins couple endosomes with Golgi during Wntless trafficking (Reshi et al., Dev Cell 2024)

In our earlier work, we found phosphatase PTEN associates with retromer components and regulates trafficking of Glut1 transporter. In addition to PTEN, several components of retromer complex being found in the interactome of other selected phosphatases. The role of these phosphatase associations with retromer complex in cells is unknown. Thus, we characterized new phosphatase-retromer associations further and found that a phosphatase complex EYA has a novel role in retrograde trafficking. Retrograde vesicular trafficking is critical for maintaining cellular homeostasis and signalling. It acts as an intersection between secretory and endocytic pathway by targeting the endosomal cargos to trans-Golgi network. Retromer complex along with associated sorting nexin proteins plays a central role in delivering cargos from endosomes to trans Golgi network. Although the specificity of cargo selection of retromer complex has

been extensively studied, the precise mechanism by which retromer complex directs the cargos specifically to TGN remains elusive. In this study, we discovered EYA complex as a molecular bridge that interacts with the retromer complex and promotes the retrograde vesicular trafficking of Wntless cargo by directing it specifically to TGN.

Although EYA proteins were known to be essential for cell-fate determination and organ development, their role in vesicular trafficking is not documented so far. We demonstrated that EYA proteins (EYA 1-4) form a hetero-tetrameric complex that interacts with the retromer complex and localizes to early endosomes. We found Wntless, a GPCR like transmembrane protein at Golgi that helps Wnt ligand anterograde movement towards the cell surface, as a cargo for EYA-Retromer complex. Depletion of EYA proteins led to defective trafficking of Wntless to Golgi leading to its accumulation at early endosomes. Subsequent to defective Wntless trafficking to Golgi, our surface microscopy using TIRF revealed a reduced Wnt3a ligand transfer to plasma membrane and secretion in EYA depleted cells. Depletion of EYA complex components severely reduced β-Catenin dependent luciferase activity in cells, clearly indicating an essential role of EYA complex in promoting Wnt signaling. To identify how EYA complex connects endosome with Golgi network during Wntless trafficking we analysed the interactome data of EYA1-4 proteins. Among different vesicular trafficking proteins that are commonly found in all EYA complexes, we demonstrated SCAMP3 to be an important EYA complex interacting partner. SCAMP3 loads onto sorting endosomes dependent on EYA complex. Similar to EYA depletion, knock down of SCAMP3 causes Wntless accumulation in the early endosomes and cargo containing vesicles derived from endosomes fail to fuse with Golgi. Wnt3a reaching the plasma membrane is significantly reduced in SCAMP3 knockdown cells, thus phenocopying the loss of EYA complex in cells. Depletion of SCAMP3 also resulted in reduced β-Catenin activity, suggesting an important role of SCAMP3 in Wnt signaling. Interestingly, by using worm, fly and zebrafish models, we found that the EYA-SCAMP3 axis is evolved in vertebrates. And we demonstrated an important clinical relevance to this complex. EYA mutations found in patients with sensorineural hearing loss forms dysfunctional EYA-retromer complex that fails to activate Wnt signaling, suggesting an important role of this complex during development. In conclusion, our study discovered a new multiprotein complex that assists retromer in determining the destination of cargos during retrograde vesicular trafficking (Summary in Figure 2) **(Reshi et al., Dev Cell. 2024).**

These systematic studies permitted us to put together an in vivo functional phosphatase network and to identify key nodules in this network as potential drug targets.

Signature of the applicant

Figure 1

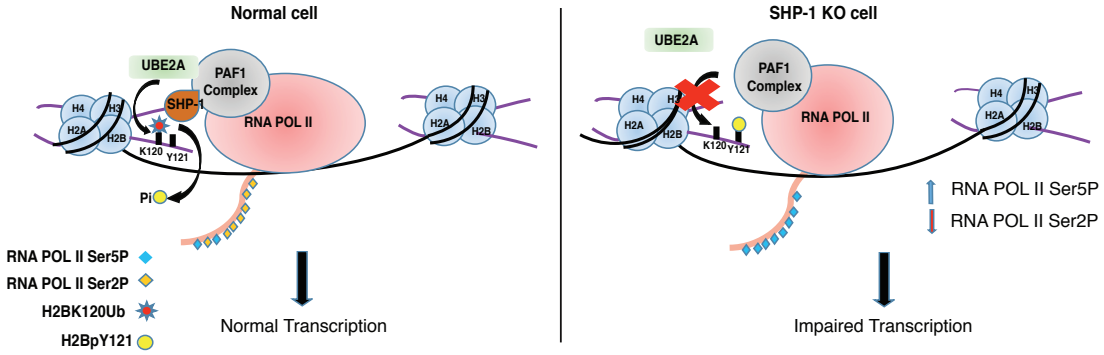
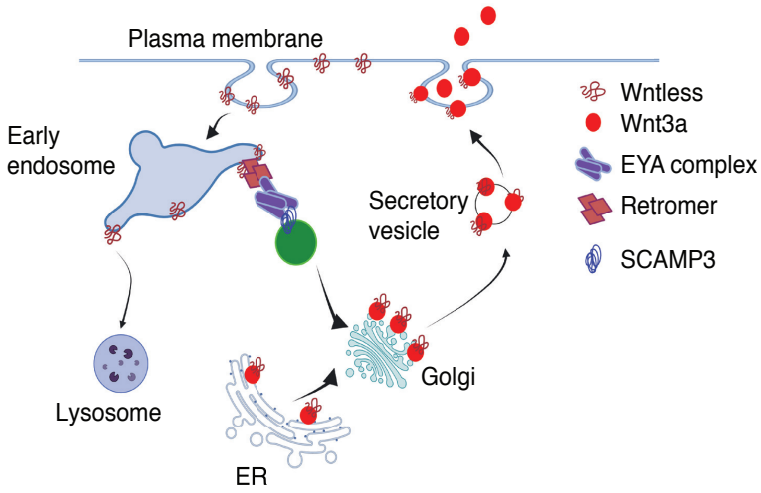


Figure 2



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