Metabolic Syndrome

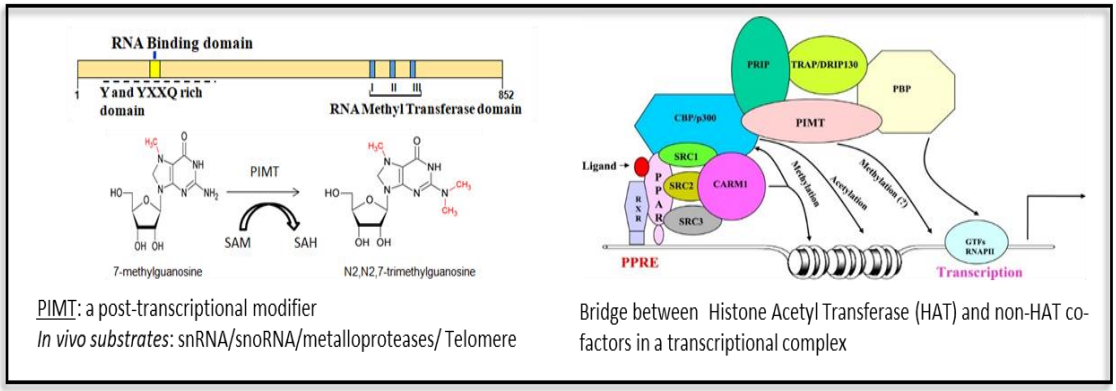
Metabolic syndrome is a cluster of different abnormalities such as insulin resistance, abdominal obesity, hypertension and dyslipidemia. It increases the risk for the development of Type 2 Diabetes and cardiovascular manifestations such as coronary artery disease. Global prevalence of metabolic syndrome is quite alarming necessitating development of better therapeutic strategies. Metabolic disease research at DRILS focusses on i) identification of novel mechanistic disease insights– identification and validation of targets ii) strategies for improved incretin mediated insulin secretion iii) pre-clinical drug discovery.

PIMT/ TGS1: PRIP Interacting protein with RNA Methyl Transferase activity- *a key driver of insulin resistance*

PIMT (PRIP-Interacting protein with Methyl Transferase activity), a co-factor binding protein, is proposed to serve as a bridge between the HAT (Histone acetyltransferases) and non-HAT co-activator complexes assembled at the gene promoters. It is also known by its synonym TGS1 (Trimethyl guanosine synthase1), which alludes to its function of m7Gcap hypermethylation of a few RNA species such as small nuclear and nucleolar RNAs (snRNAs and snoRNAs), selenoprotein, and telomerase RNAs.

PIMT/TGS1 is involved in pre-mRNA splicing, transcription, and ribosome biogenesis. 1) Using liver-specific PIMT knockout mice (PIMTD Liv KO mice), Dr. Parimal's group earlier reported that glucose release was significantly reduced in PIMTD Liv KO hepatocytes as compared to PIMTfl/fl hepatocytes, demonstrating that PIMT regulates hepatic glucose output. It was also showed that PIMT is up-regulated in rats fed a high-sucrose diet (HSD). HSD leads to chronic systemic inflammation, leading to elevated levels of circulating TNFα. The expression of PIMT is up-regulated upon the exposure of myoblasts or myotubes to TNFα, resulting in impaired insulin-stimulated glucose uptake. Further Dr. Parimal's team has shown that ablation of PIMT promotes nuclear translocation of insulin in the pancreatic β-cells, PIMT regulates pro-inflammatory macrophage mediated paracrine insulin resistance through cross talking between macrophages and skeletal muscle cells and inhibition of PIMT in adipose tissue leads to insulin sensitization and body weight loss. 2) A new mechanism of how statins, the widely used cholesterol-lowering drugs may cause insulin resistance and diabetes by increased fatty acid accumulation within the muscle cells, thus impairing insulin signalling and how statins enhance the diabetes in the hyperglycaemic, hyperlipidemic and obese animals was described and 3) contributed to discover the 1st in class small molecule inhibitor of Chorismate

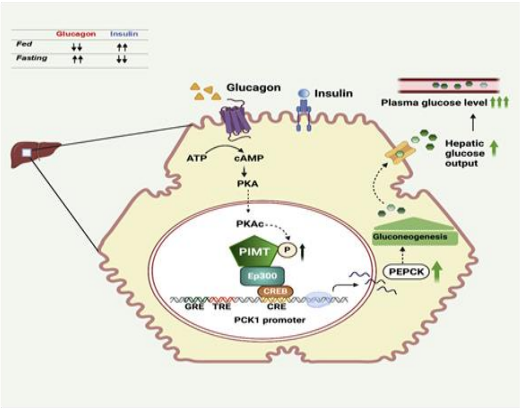
Mutase (CM) and establishment of Proof of Concept in animal for the treatment of latent, replicating and MDR TB.



PIMT: a post-transcriptional modifier
In vivo substrates: snRNA/snoRNA/metalloproteases/ Telomere

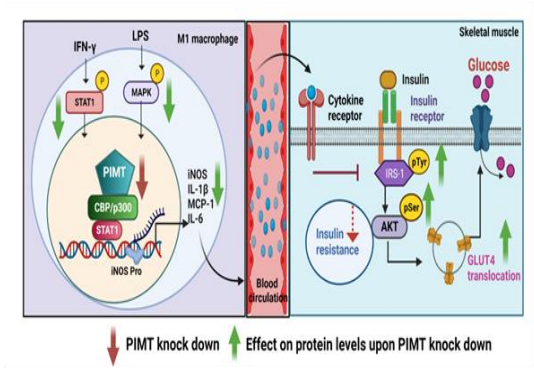
Bridge between Histone Acetyl Transferase (HAT) and non-HAT co-factors in a transcriptional complex

PIMT regulates hepatic gluconeogenesis in mice

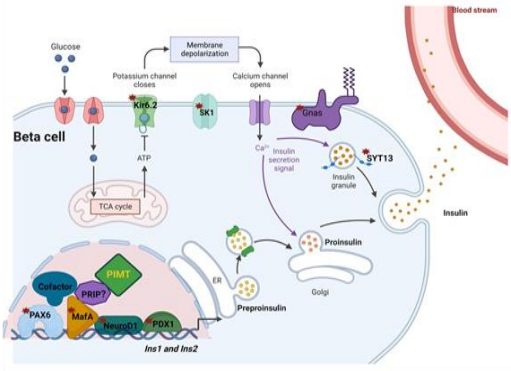


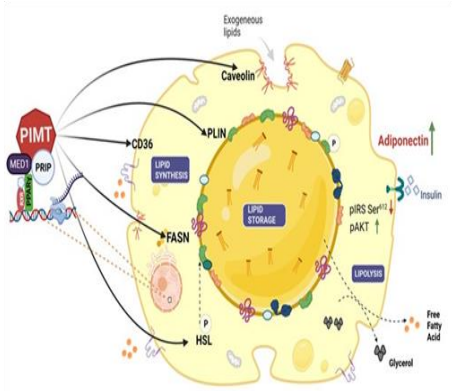
PIMT Controls Insulin Synthesis and Secretion through PDX1

PIMT regulates pro-inflammatory macrophage mediated paracrine insulin resistance



PIMT knockdown reduces lipid accumulation in adipocytes, limits body weight gain and promotes insulin sensitivity in mice





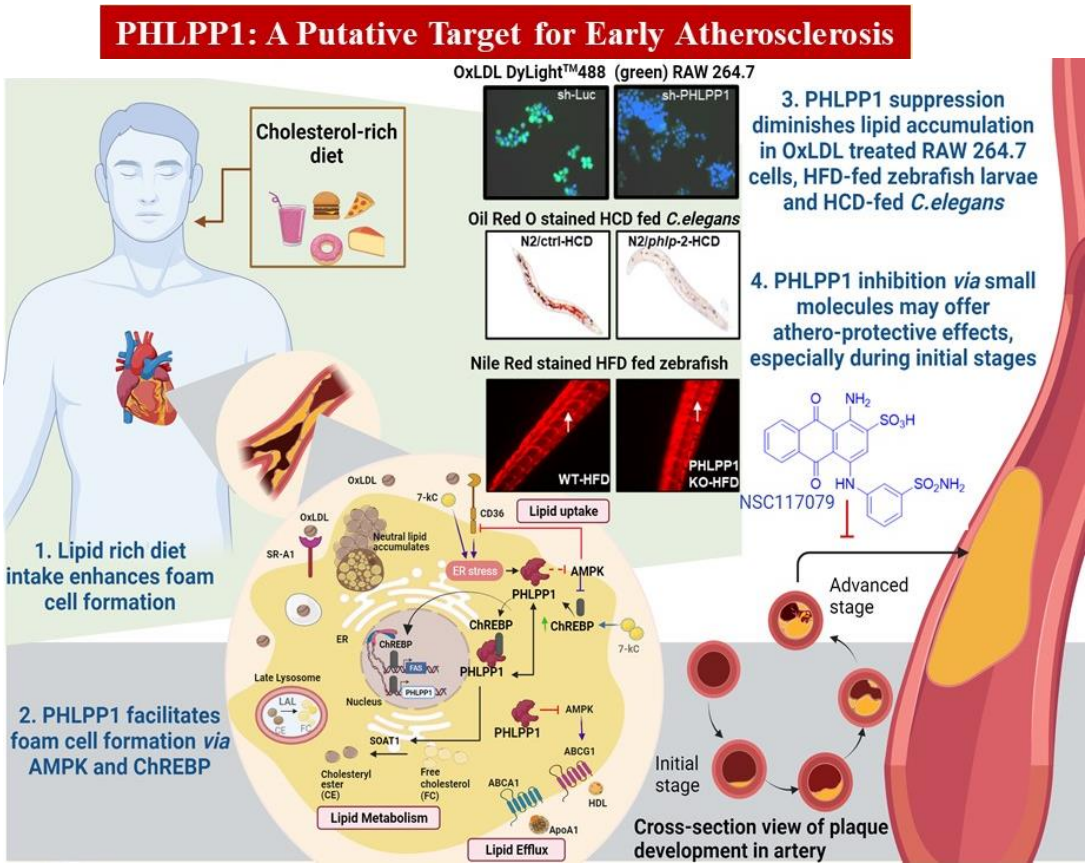
Probing the functional significance of Ser/Thr Phosphatase, PHLPP1, in metabolic syndrome

PHLPP1 (PH domain and Leucine rich repeat Protein Phosphatase 1), belonging to a novel family of Ser/Thr phosphatases, was identified in 2005 during a search of human genome for a phosphatase containing a pleckstrin homology (PH) domain, and was named to reflect its domain composition. The most well studied aspect of PHLPP1 is its tumour suppressor role,

and its importance in other biological processes, especially in the different facets of metabolic syndrome, is being explored. Past studies from Dr. Kishore Parsa's laboratory, implicated PHLPP1 in the control of macrophage mediated inflammation and skeletal muscle insulin resistance. The following are recent studies from Dr. Parsa's laboratory, which uncover its involvement in other aspects of metabolic abnormalities.

Role of PHLPP1 in the formation of foam cells

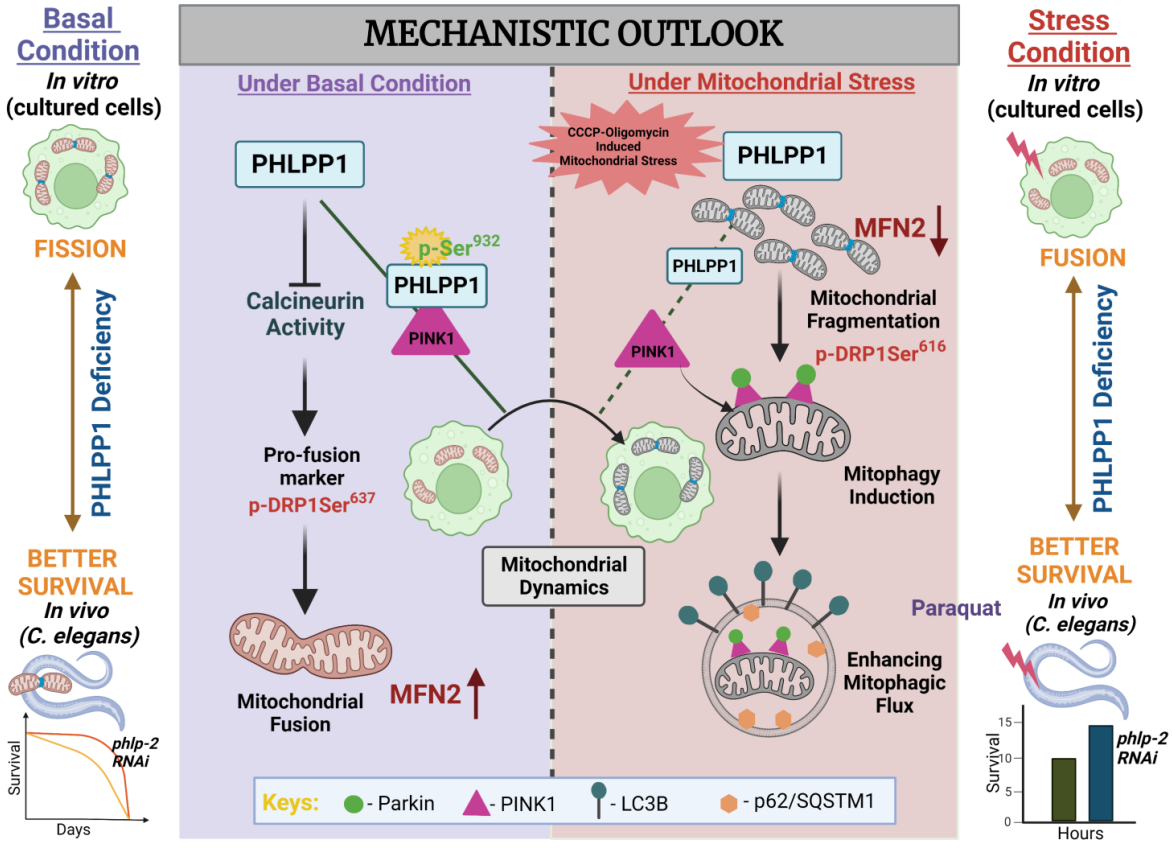
Atherosclerosis is a chronic inflammatory vessel disease occurring due to the accumulation of lipids in the arterial wall. It is the principal cause of cardiovascular diseases (CVD), the leading killer worldwide. Despite the management of lipids with current medications, there is a strong unmet medical need for the treatment of atherosclerosis necessitating development of newer and better therapies. The infiltration of foamy macrophages into the arterial intima is a distinctive characteristic of early atherosclerotic lesions. Foam cells are lipid-laden macrophages, which are formed due to disruption of the cholesterol handling pathways. Earlier, we reported that PHLPP1 restrained the pro-inflammatory responses of macrophages induced by bacterial endotoxin and IFNγ. In this study, we explored the potential involvement of PHLPP1 in the lipid accumulation in macrophages. In this direction, we observed that levels of PHLPP1 were elevated in macrophages exposed to oxidized-LDL and in zebrafish larvae fed with a high-fat diet (HFD). Through overexpression and knockdown techniques, we demonstrated that PHLPP1 contributes to the accumulation of neutral lipids, leading to increased cellular levels of total cholesterol and free fatty acids (FFAs). Transcriptomic analysis revealed the involvement of PHLPP1 in lipid metabolism, cholesterol biosynthesis, inflammatory pathways etc. Furthermore, we found that PHLPP1 interacted with and enhanced the recruitment of ChREBP, a glucose responsive lipogenic transcription factor, to the promoter of *Fasn*, which is involved in fatty acid synthesis. The lipid accumulation in macrophages mediated by PHLPP1 was mitigated by the activation of AMPK, a kinase, which promotes lipid catabolism. Both pharmacological inhibition and CRISPR/Cas9-mediated disruption of PHLPP1 resulted in reduced lipid accumulation in the intersegmental vessels of HFD-fed zebrafish larvae, accompanied by a decrease in total cholesterol and triglyceride levels. Additionally, the deficiency of phlp-2, the *C. elegans* ortholog of PHLPP1/2, abrogated lipid accumulation in worms exposed to a high-cholesterol diet. In conclusion, our findings suggest that PHLPP1 plays a significant role in promoting lipid buildup and in the early stages of atherosclerosis.



Impact of PHLPP1 on mitochondrial homeostasis

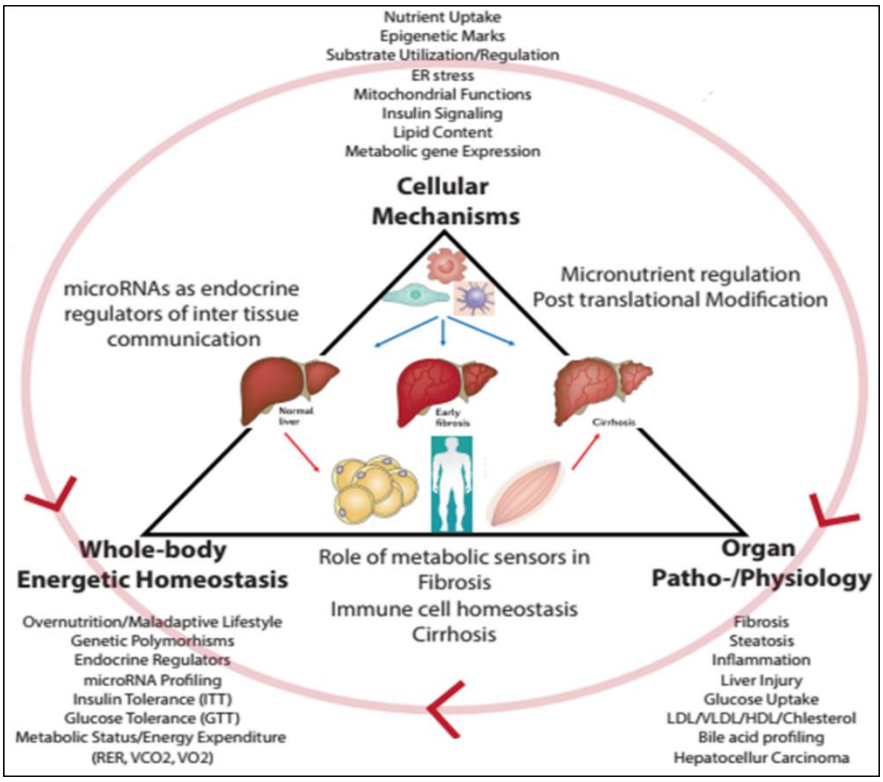
Adaptability to both intracellular and extracellular signals is crucial for maintaining cellular homeostasis. The intricate control of mitochondrial morphology and functions by metabolic signals is vital for regulating bioenergetics and metabolism. Dysfunctional mitochondria are associated with abnormal mitochondrial dynamics, affecting diverse metabolic pathways. We have earlier showed that PHLPP1 promotes endoplasmic reticulum stress, consequently, skeletal muscle insulin resistance, through the inactivation of the energy sensor, AMPK. Considering the close association between ER and mitochondria, anatomically and functionally, in this study, we explored the involvement of PHLPP1 in the maintenance of mitochondrial homeostasis. Mitochondrial shape and functions are controlled by the opposing events of elongation and fragmentation of mitochondria. We observed that PHLPP1-depleted HEK 293T and C2C12 myoblast cells presented an enhanced globular mitochondrial structure, while the ectopic expression of PHLPP1 resulted in mitochondrial tubularity. Through overexpression and knockdown strategies, we demonstrated that PHLPP1 influences the protein levels of mitochondrial pro-fusion markers MFN2 and p-DRP1Ser^{637} levels. Conversely, under mitochondrial stress, PHLPP1 induced mitochondrial fragmentation by

increasing mitochondrial pro-fission markers, total DRP1, and p-Drp1Ser^{616}. At the molecular level, we observed that PHLPP1 interacted with and dephosphorylated calcineurin, a p-DRP1Ser^{637} phosphatase, under basal conditions. Additionally, we showed that PHLPP1 formed dimers with PINK1 under basal conditions, but such interaction was disrupted upon CCCP and oligomycin-induced mitochondrial stress. Intriguingly, upon mitochondrial membrane depolarization, we observed that PHLPP1 facilitated PINK1 stabilization, parkin recruitment to mitochondria, and induced the activation of the mitophagy machinery. This provides a molecular explanation for the dual effects of PHLPP1 on mitochondria under different conditions. Consistent with our *in-vitro* findings, we found that the depletion of phlp-2, the *C. elegans* ortholog of PHLPP1, resulted in mitochondrial fission under basal conditions, extended the lifespan of the worms, and enhanced their survival when exposed to paraquat-induced oxidative stress.



Understanding the role of nutrient-dependent cellular mechanisms in regulating immune-metabolic homeostasis in the liver, with emphasis on metabolic-dysfunction associated fatty liver disease (MAFLD).

Work over several decades suggest that liver acts as the main hub for the orchestration of cellular and physiological responses to various dietary and metabolic conditions. Liver dysfunction is often associated with metabolic pathologies such as obesity, non-alcoholic fatty liver disease (NAFLD), insulin resistance, diabetes, atherosclerosis and cardiovascular disease. Both acute and chronic liver dysfunction represents a major global health burden and an important cause of morbidity and lethality in India and worldwide, with widespread socio-economic consequences. Studies show that liver dysfunction is preceded by inflammation, and elements of both the innate and adaptive immune systems are pivotal in both initiating and regulating the disease progression.



Dr. Tandrika's interest lies in investigating the mechanism of cross-talk between nutrition and chronic activation of tissue-associated immune cells. Her work entails investigation into the cellular mechanisms that mediate the resolution of fibrosis and restoration of tissue homeostasis, and how diet dependent alterations in liver metabolism rewires tissue resident macrophages, thereby contributing towards liver pathology. Many lines of evidence indicate that chronic over-nutrition causes metabolic abnormalities and predispose patients to non-alcoholic fatty liver disease (NAFLD), which eventually puts them at risk of developing cardiovascular diseases (CVD) and diabetes.

However, the cellular mechanisms underlying the ensuing caloric imbalance remain poorly understood. Understanding such pathways will provide us with improved alternatives to current therapeutic interventions, hopefully decreasing the burden of these maladies. Therefore, the 3 main focal points of research interests of Dr. Tandrika's group includes: (1) Investigating nutrient/diet dependent regulation of cellular mechanisms (epigenetic, transcriptional and signalling) maintaining metabolic homeostasis with emphasis on liver physio/patho-physiology; (2) Bidirectional relationship between the heterogeneous cellular milieu regulating energy intake and utilization – Role of Immune Cell homeostasis and (3) Inter-organ communication via endocrine/secreted mediators.