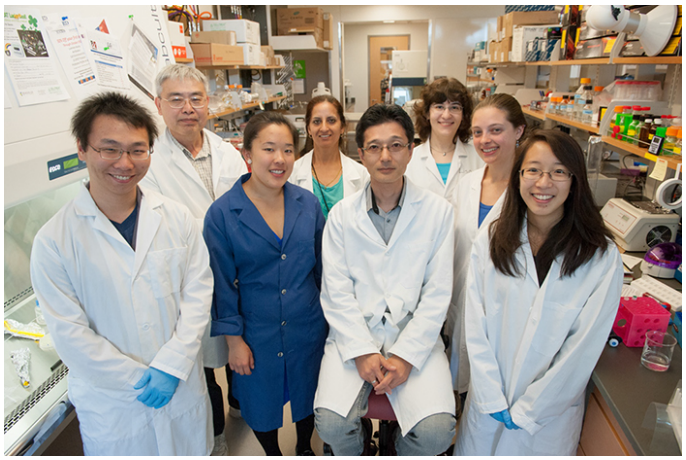
Principal Investigator: Yasu S. Morita

Grant Title: Primordial endoplasmic membrane in bacteria with roles in glycan biosynthesis

Abstract:

Mycobacterial cell envelope has a complex structure, composed of a waxy outer membrane linked to the glycan-rich core, and an innermost plasma membrane containing unique phosphatidylinositol (PI)-anchored glycans. Importantly, mycobacteria grow from their cell poles, implying that the synthesis of cell envelope glycans and glycolipids requires spatiotemporal regulation. We previously reported that *Mycobacterium smegmatis* cell lysate can be fractionated into two distinct membrane fractions: the PM-CW, which is a plasma membrane tightly associated with the cell wall, and the PMf, which is a pure membrane free of the cell wall components. Interestingly, the early and late steps of PI-anchored glycan biosynthesis are compartmentalized into the PMf and PM-CW membrane fractions, respectively, suggesting a spatial regulation of cell envelope biogenesis. The ***objective*** of this study was to determine the function and subcellular localization of this novel bacterial membrane in live mycobacterial cells. We first performed comparative proteomics of these two membrane fractions, revealing that the PM-CW is a canonical plasma membrane fraction containing proteins such as transporters, sensor kinases, and enzymes involved in the respiratory chain. In contrast, the PMf is bound by enzymes for the synthesis of lipids, glycans and other metabolites, including PimB', a mannosyltransferase involved in the biosynthesis of PI-anchored glycans, and PyrD, an enzyme involved in pyrimidine biosynthesis. Using immunoprecipitation and negative staining immuno-electron microscopy, we then showed that two of such PMf-associated proteins are bound to the same membrane vesicles. Finally, using fluorescent microscopy, we showed that the PMf membrane is enriched in the growing poles of mycobacterial cells. Taken together, our data suggest that the PMf is a growth pole-associated biogenic membrane with distinct roles in cell envelope biogenesis.



From left: David Luo, Tsungda Hsu, Jennifer Hayashi, Manju Sharma, Yasu Morita, Lisa Baumoel, Samantha Giffen and Deborah Lee.