Chapter 7

Abstract

Mycobacterium tuberculosis (Mtb) is the bacterial pathogen that causes the infectious disease tuberculosis. Novel therapies are needed to combat the spread of *Mtb*, as it has developed resistance to current treatments. The characteristic lipid-rich cell wall composition and ability of *Mtb* to shift from active growth to the latent state are two major challenges to the development of new therapies. The membrane phospholipids (PL) of *Mtb* include several acidic species such as phosphatidylglycerol (PG), cardiolipin, phosphatidylinositol and its mannoside derivatives, and a single basic species, phosphatidylethanolamine. Here we demonstrate that an additional basic PL, lysinylated PG (L-PG), is present in *Mtb* H37Rv and that the *lysX* gene encoding the two-domain lysyl-transferase (mprF)-lysyl-tRNA synthetase (lysU) protein is responsible for its production. The *Mtb lysX* mutant is sensitive to cationic antibiotics and peptides, shows increased association with lysosome-associated membrane protein–positive vesicles, and it exhibits altered membrane potential compared to wild type. A *lysX* complementing strain expressing the intact *lysX* gene, but not one expressing the *mprF* domain alone, restored the production of L-PG and rescued the *lysX* mutant phenotypes, indicating that the expression of both proteins is required for

LysX function. The *lysX* mutant also showed defective growth in mouse and guinea pig lungs and showed reduced pathology relative to wild type, indicating that LysX activity is required for full virulence.

We show that a *lysX* complement strain expressing *lysX* from the inducible tet promoter is proficient in complementing *lysX* phenotypes confirming that the observed phenotypes are specific to *lysX*. To evaluate the correlation between changes in membrane potential and *lysX* activity, we visualized regions of cardiolipin (CL), one of the abundant phospholipids of mycobacteria, by staining with fluorescent dye 10-N-nonyl acridine orange (NAO) and found that CL is localized as bright spots at septal regions and poles of actively dividing cells, but not in stationary phase cells. The lysX mutant was elongated and showed more numerous and brighter CL staining at both midcell and quarter cell septa, compared with wild type, indicating a defect in the cell division process. Evaluation of 14C-acetic acid incorporation into major phospholipids such as CL, phosphatidylethanolamine (PE), phosphatidylinositol, and their degradation between *lysX* mutant and its parent revealed differences in the turnover of PE and PI.

Further examination of NAO staining revealed that cells with defects which disrupt septa formation lack staining patterns associated with the presence of CL enriched regions. The distribution of NAO staining patterns shift to reflect synchonous growth in a cold-sensitive *Mtb* strain in which the cell cycle has been disrupted and allowed to restart again at a permissive

temperature. Localization of the replication initiation protein DnaA was similar to the location of CL enriched regions in cell membrane, indicating that this protein may interact with CL at midcell, quartercell or cell poles.

Together, our results suggest that LysX-mediated production of L-PG is necessary for the maintenance of optimal membrane integrity and for survival of the pathogen upon infection. Our data also favor a hypothesis that alterations in phospholipid metabolism could be contributing to changes in membrane potential, hence the observed phenotype of lysX mutant. Moreover, the organization and localization of membrane phospholipids may provide clues to function of cell cycle proteins and their interaction with organized lipid domains.