Investigating the coordinated regulation of lipopolysaccharide (LPS) and phospholipid amounts by the essential LPS assembly proteins LapB/LapC and the involvement of a new thioesterase

The most defining and distinguishing feature of Gram-negative bacteria, such as *Escherichia coli*, is the presence of an asymmetric outer membrane (OM), which is essential for their viability. This asymmetry is due to the presence of lipopolysaccharide (LPS) in the outer leaflet of OM and phospholipids facing the inner leaflet. LPS is one of the major virulence factors in pathogenic bacteria and is the causative agent of bacterial sepsis. Globally, sepsis is one of the leading causes of mortality, with more than 1500 deaths per day. LPS covers approximately 70% of the cell surface, and its synthesis and translocation to the OM require more than 50 genes, out of which approximately 30 are essential (10% of total essential genes in *E. coli*). As many of them are essential and unique to bacteria, they are validated targets for the discovery of new antibiotics and vaccines. For balanced bacterial growth, a tight balance exists between phospholipids and LPS as they share a common metabolic precursor. Any imbalance in the ratio of LPS vs phospholipids causes bacterial lethality. This balance is achieved by the regulation of the amounts of LpxC, which catalyzes the first committed step in LPS biosynthesis, and the FabZ enzyme that initiates the phospholipid synthesis. LpxC is known to be an unstable protein, with its stability regulated by several factors. Over the past several years, we have shown that FtsH and LapB mediate LpxC proteolysis and that the newly discovered essential LapC protein inhibits LapB activity and prevents unwanted LpxC removal. Since it is unknown how the activity of LapB and LapC is regulated in response to LPS demand, and which amino acid residues in LapB and LapC mediate interaction with LPS, we investigate these aspects in the project. The LPS of wild-type *E. coli* strains contains the highly conserved hexaacylated lipid A (endotoxin principal), which is recognized by the essential LPS transporter MsbA for its translocation across the inner membrane (IM). Our recent studies revealed that when bacteria synthesize underacylated LPS, they require cardiolipin for their viability. Similarly, in the absence of LapD and/or dysfunctional LapC, the cardiolipin synthesis is also essential, although the molecular basis of such a requirement is not understood. To address these critical gaps, several approaches are taken. Firstly, mutagenesis of LapB and LapC has been initiated, and mutant proteins that seem to have lost the ability to interact based on pull-down experiments suggest that transmembrane anchors of LapC and the N-terminal LapB membrane region mediate their interaction. We plan to carry it forward and identify specific amino acid residues that are required for LPS binding and protein-protein interactions, which will be established by measuring binding affinities and their impact on LpxC proteolysis. Taking advantage of the severe LPS-associated growth defects of strains with impaired LapC and the conditional lethal phenotype of strains simultaneously lacking cardiolipin synthase and the last enzyme that generates hexaacylated lipid A, suppressors were isolated that overcome their lethality to further elaborate our knowledge about LpxC regulation and how bacteria maintain OM symmetry. Our initial results identified another new protein TesD with a predicted thioesterase activity. Based on initial biochemical studies, TesD was found to interact with several enzymes involved in LPS and phospholipid synthesis, including the central cofactor acyl carrier protein. Thus, the proposed experiments will quantify these interactions as well as implore the impact on LpxC stability and LPS content, when either TesD is absent or overproduced. Furthermore, we aim to measure if TesD has selectivity towards specific acyl chain length of fatty acids that may impact on the balance between saturated vs. unsaturated fatty acids, which can alter LpxC stability. Suppressors that restore the growth of bacteria with impaired LapC will be isolated and tested to determine if they restore OM asymmetry and suppress permeability defects. Experiments will be undertaken to address if their overexpression prevents hyperdegradation of LpxC in *lapC* mutants or acts as an inducer to signal LpxC stabilization. Overall, these experiments will together identify novel regulatory controls of LPS and phospholipid biosynthesis. Finally, peptides based on LapB and LapC can have the potential to serve as new antimicrobials in the future. LpxC inhibitors, such as CHIR090, are already known new antibiotics and a peptide from LapC has also been shown to be effective against certain bacteria, hence our studies will have a broader impact.