UNDERSTANDING THE ROLE OF CARDIOLIPIN IN HELICOBACTER PYLORI FLAGELLAR SYNTHESIS

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ABSTRACT

*Helicobacter pylori* is an Epsilonproteobacteria that colonizes the human stomach mucosa and a causative agent of peptic ulcers and gastric cancer. *H. pylori* uses a cluster of polar, sheathed flagella for motility, which is required for host colonization. As part of my dissertation studies, I found the glycerophospholipid cardiolipin (CL) has a role in flagellum biogenesis. Inactivating a cardiolipin synthase gene (*clsC*) in *H. pylori* G27, but not *H. pylori* B128, abolished flagellum biosynthesis. Analysis of glycerophospholipids in the two *clsC* mutants revealed that both were similarly deficient in CL. Motile variants of the G27 *clsC* mutant were obtained following transformation with genomic DNA from the B128 *clsC* mutant. Resequencing the genomes of seven motile G27 *clsC* recipients showed that all possessed the *flgI* (encodes flagellar P ring protein) allele from B128. The G27 and B128 FlgI proteins differ at five amino acid positions. Introducing the B128 *flgI* allele into the G27 *clsC* mutant rescued flagellum biosynthesis. Cryo-electron tomography (cryo-ET) of the G27 *clsC* mutant showed flagellum assembly was arrested at a step that proceeded formation of the P ring. Taken together, these results suggest G27 FlgI, but not B128 FlgI, fails to form the P ring in the absence of wild-type CL levels. In related studies, I discovered CL appears to be a major component of the *H. pylori* flagellar sheath, a membrane that surrounds the flagellum and is contiguous with the outer membrane. Using a comparative genomics approach, I identified several genes that are present in *Helicobacter* species that possess a sheath but are absent in *Helicobacter* species that lack a sheath. Interestingly, one of these genes is *clsC*. Four other sheath-specific genes (HP1486-HP1489) encode a predicted efflux system that I targeted for mutagenesis. Disrupting the HP1488 homolog in B128 inhibited flagellum biosynthesis. Cryo-ET revealed that the B128 mutant was missing part of a cage-like structure that surrounds the flagellar motor. I hypothesize the cage-like structure is an efflux system encoded by HP1486-HP1489 that transports CL to the outer membrane where it is incorporated into the flagellar sheath.

INDEX WORDS: *Helicobacter pylori*, flagella, ClsC, RpoD, RpoN, FliA, FlgI