Examining the glycosylation pathway in *Helicobacter Pylori* using mass spectrometry
Catherine Liu, 2019

The pathogenic bacterium *Helicobacter pylori* (*Hp)* is responsible for duodenal ulcers as well as stomach cancer, the second leading cause of cancer death worldwide (1). However, there is growing concern for *Hp's* ability to resist current antibiotic treatments (2). In addition, current antibiotic treatments are non-specific for pathogens, and kill the advantageous stomach bacteria (2). Thus, there's a need to develop new treatments that will specifically target *Hp.*

The glycoproteins in *Hp* represent an intriguing target for therapeutics, since their glycan structures are distinctive from their eukaryotic counterparts (3). They also often play an important role in pathogenesis. Pathogenic bacteria that cannot synthesize glycoproteins have shown a range of defects, including reduced adhesion to host cells, defective biofilm formation, and disrupted flagellin formation, leading to an immotile pathogen that cannot colonize the host (4, 5). Thus, understanding the glycosylation pathway and the enzymes involved can help further elucidate how to target these glycoproteins.

Previous work in the Dube lab has identified 13 *Hp* glycosyltransferase genes required for glycoprotein biosynthesis. To achieve this goal, the Dube lab developed 13 *Hp* G27 mutant strains containing insertions in these glycosyltransferase-encoding genes. These thirteen mutants displayed reduced glycoprotein production compared to wild type, and intriguingly, there was notable similarity of glycoprotein labeling across all mutant strains (6). These results indicated a common biosynthetic pathway, and indicated that these 13 *Hp* genes were involved in this pathway. However, the mechanisms of the glycosylation pathway and how these enzymes work together is still unknown. Understanding the mechanisms of this pathway will shed light on understanding how glycan structure relates to its function in pathogenesis, as well as enable the development of novel glycosylation-based drug targets.

This summer, I sought to understand the glycosylation pathway by using mass spectrometry as an analytical tool. Mass spectrometry can fragment the bonds within a chemical species, and measure the masses of the resulting fragments. Since glycans tend to fragment along the bonds that connect their monosaccharide building blocks, and these units have characteristic masses, we can use mass spectrometry data to potentially build up the entire glycan structure. Using mass spectrometry, I hope to examine the glycan structures on the 13 *Hp* G27 mutant strains as well as on wild-type *Hp*, and I hypothesize the mutant strains will contain truncated glycans compared to wild type. The extent of glycan complexity will reveal the role of each glycosyltransferase enzyme, and determine its relative placement in the enzymatic pathway. Thus, using mass spectrometry analysis of glycan structure, I hope to piece together the full model of glycan assembly.

I began by establishing a method to examine glycan structure in *Hp* using mass spectrometry. My approacwuss spectrometry. My

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