Newly Discovered Cell Shape Promoting Protein Complex Involved in the Maintenance of Distinct Helical Shape of *Helicobacter Pylori*

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ABSTRACT

The distinct helical shape of the bacterium *Helicobacter Pylori (H. pylori)* assists this organism in colonizing the digestive organs of its target host. It has been discovered that a key determinant of helical cell shape formation in *H. pylori* is the Csd5 protein, which engages in multiple cell shape promoting interactions with the cell wall and other various proteins. This finding has significant clinical implications, as it outlines Csd5 as a potential drug target for treating *H. pylori* infection in the future.

Keywords: *H. pylori*, Csd5, cell shape, protein complex, drug target

Bacterial infections of *H. pylori* in the stomach have been shown to greatly increase the likelihood of developing debilitating diseases such as stomach cancer.[1] A successful infection is dependent upon the organism's ability to colonize the stomach and the digestive tract.[2] In *H. pylori*, this process is aided through its unique helical cell shape which helps it penetrate the mucoid lining of the stomach to allow for successful colony formation.[3] Therefore, investigating the mechanisms that contribute to cell shape formation is of great interest to researchers as this knowledge has the potential to be applied in a therapeutic setting.

Bacterial cell shape is determined by the cell wall, which is predominately composed of carbohydrate molecules known as peptidoglycan (PG).[4] This molecule has a chain-like structure consisting of alternating N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) monomers.[4] In the cell wall, these chains are crosslinked together for added structural support (Figure 1A).[4] It has been observed that during bacterial growth, specific intracellular mechanisms are activated to successfully form the desired cell shape via chemical interactions with the PG cell wall.[5]

With regards to *H. pylori,* a fundamental component of helical cell shape formation is the protein Csd5, which is a non-enzymatic protein unique to the species.[2] This protein was previously characterized in a study by Dr. Laura Sycuro et al. (2012), where it was discovered to be integral to the construction of helical cell shape in *H. pylori.* Specifically, it was found that cells containing a chromosomal deletion of the csd5 gene elicited a rod-shaped phenotype instead of the helical shape characteristic to *H. pylori.*[5] Additionally, PG levels within these cells remained unchanged, which suggested that alteration of cell shape stemmed from structural changes to existing PG within the cell. On the basis of these observations, it was thought that Csd5 acted alongside other Csd class proteins (namely Csd4), to promote the development of helical cell shape formation through the modification of PGs.[5] The specific mechanisms through which this occurred, however, remained uncharacterized. Consequently, this study laid the foundations for subsequent researchers to further investigate the properties of Csd5 in order to better understand the various mechanisms underlying structural phenotype in *H. pylori.*

This unique cell shape determining mechanism was the focus of a 2018 study by Dr. Kris Blair et al. from the University of Washington, who was assisted by Dr. John Whitney, a prominent researcher from Michael DeGroote Institute for Infectious Disease Research at

McMaster University. In this study, it was initially hypothesized that Csd5 was involved in the formation of helical cell shape through its ability to promote the alteration of crosslinking between PGs in the cell wall. Specifically, this enzyme was thought to be able to localize PG carboxypeptidases which could subsequently promote or inhibit downstream PG hydrolases to alter the crosslinking of PGs.[2] This hypothesis, however, was ultimately rejected due to the lack of reproducibility in *in vivo* studies.[2] Instead, it was found that Csd5 induces helical shape by interacting with the cell wall and various other proteins to form a cell shape promoting complex.[2]

To conduct this study, the researchers first analyzed Csd5 in order to identify any important structural features of the protein. This was done using Jackhammer, which is a computerized sequencing technology designed to predict functional domains and motifs.[2] The analysis uncovered the presence of highly conserved N-terminal and C-terminal domains, as well as a less conserved, disordered domain within the middle of the protein.[2] Furthermore, it was discovered that the N-terminal region was responsible for mediating protein interactions while the C-terminal region was found to contain a unique SRC Homology 3 (SH3) domain that directly interacted with peptidoglycans.[2] Additionally, it was seen that the disordered domain contributed to the ability of the protein to maintain proper curvature and axis length of the membrane.[2]

After the characterization of the Csd5 protein, the researchers set out to further investigate the specific interactions at each domain. Upon analyzing the SH3 domain by homology modelling, a conserved functional motif within this domain, known as the Reverse Transcription (RT) loop, was discovered. Interestingly, this motif was found to be homologous to peptidoglycan binding motifs in other bacterial species.[2] Using this homology model, the researchers identified amino

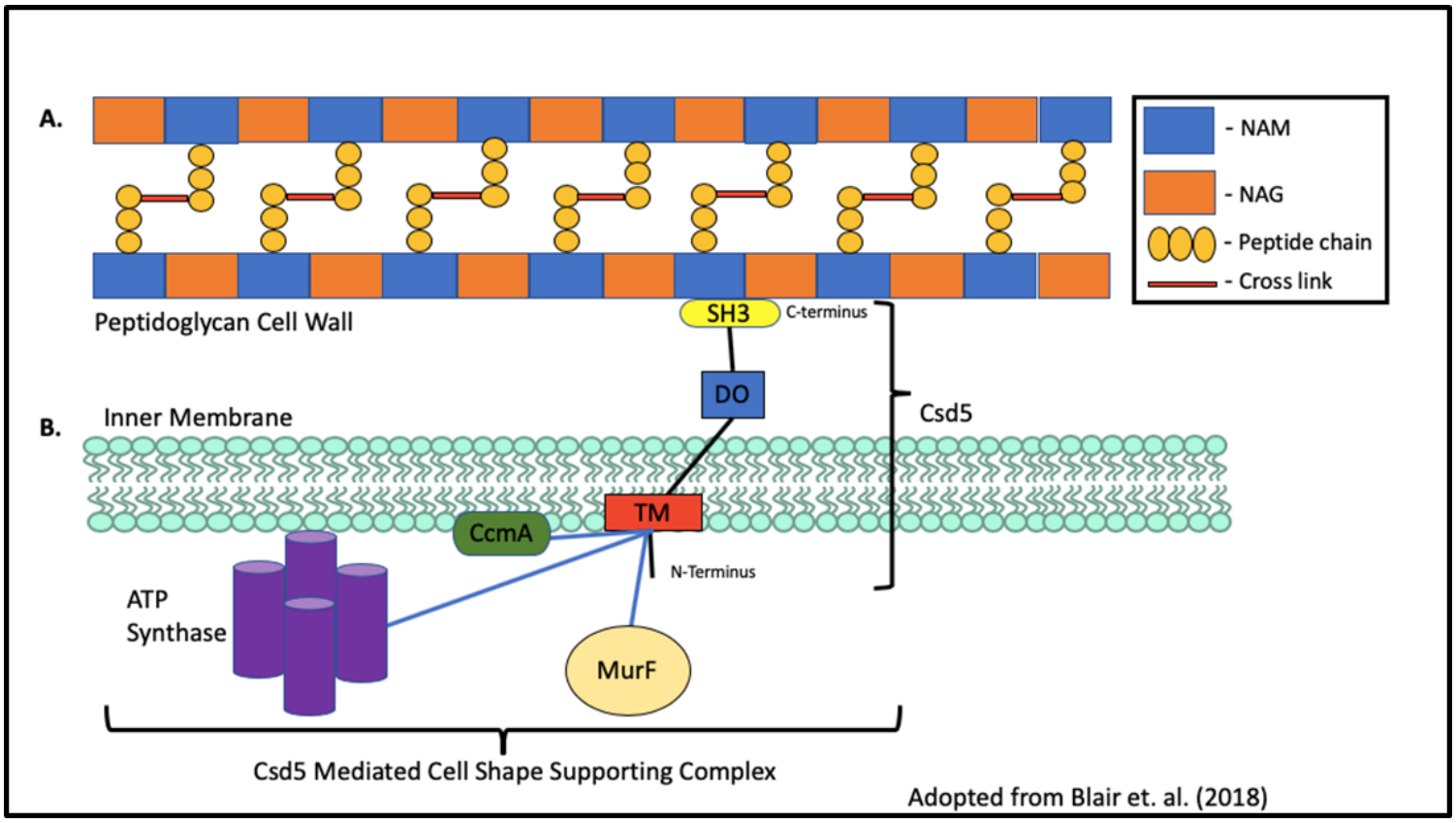

Figure 1A. Schematic showing the structural composition of the bacterial cell wall. The cell wall is positioned between the inner cell membrane and the outer cell membrane (not demonstrated). It is composed of alternating NAM and Nag residues that are crosslinked via peptide chains that extend from NAM residues. **Figure 1B. Hypothesized model for *H. pylori* cell shape promoting complex mediated by Csd5.** RT loop motif mediates peptidoglycan binding of the highly conserved C-terminal SH3 domain of Csd5. The disordered domain in the middle of Csd5, containing a characteristic glutamine-rich coiled-coil motif, helps to maintain cell axis length and curvature. The highly conserved N-terminal domain of Csd5 promotes interactions (represented by the blue lines) with the peptidoglycan synthesis enzyme MurF, the cell shape protein CcmA, and the bacterial ATP synthase. Overall, it is proposed that this multiprotein complex, composed of Csd5, MurF, CcmA and ATP synthase, is involved in maintaining the helical shape of *H. pylori*.

acids within the RT loop believed to be essential for binding, specifically, Arg146 and Thr151.[2] Consequently, when mutations were introduced at these sites, the helical cell structure was disrupted.[2] This confirmed the importance of this motif for interacting with peptidoglycans.

Next, the researchers used a proteomic screen to identify the distinct proteins interacting with Cds5 at its N-terminal domain. Through this screen, three candidate proteins were identified: MurF (a cytosolic peptidoglycan precursor enzyme), CcmA (an *H. pylori* cell shape protein), and ATP synthase.[2] In order to confirm these results, deletions were introduced into the N-terminal domain to verify that this region was, in fact, responsible for mediating interactions with the candidate proteins.

In the end, the researchers found that N-terminal deletions resulted in the disassociation of these proteins from Cds5, which evidently confirmed the function of this region.[2] Another notable finding that arose was the presence of a transmembrane domain at the N-terminus. It was observed that this domain helped to anchor the N-terminus of Csd5 into the inner membrane of the cell while simultaneously promoting interactions with the candidate proteins.[2]

Overall, the results from the study by Blair et al. (2018) clarified the role of Csd5 in mediating the formation of the helical shape of *H. pylori*. Specifically, these findings provide evidence for a multi-protein complex that exists within this microbe which induces and maintains helical shape.[2] A schematic of this proposed complex can be seen in Figure 1B.

The findings by Dr. Blair et. Al. are incredibly significant as they help to foster a more comprehensive understanding of the complicated mechanisms used by *H. pylori* to regulate cell shape. Consequently, this knowledge has the potential to be clinically beneficial as it could advance researchers to develop antibiotics against *H. pylori* that are designed to disrupt its cellular shape. As stated previously, the distinct helical shape of *H. pylori* facilitates the invasion and infection of host organisms.[2] Therefore, compounds which alter the shape of this bacterium may be effective in treating conditions related to *H. pylori* infection.

Currently, one of the frontline treatment methods for these types of infections is the antibiotic Clarithromycin, which is a ribosomal inhibitor in bacterial cells.[6] While this compound has been effective in the past, Clarithromycin-resistant *H. pylori* infections are becoming more common in patients and consequently pose a significant clinical challenge.[6] Based on the evidence put forward by Dr. Blair et al., a promising next step would be to investigate Csd5 as a drug target. Due to the role that Csd5 plays in determining cell shape, successful inhibition of this protein may disrupt the ability of *H. pylori* to colonize the digestive tract and subsequently prevent infection. Ideally, more research investigating Csd5 and cell shape disrupting antibiotics should be conducted to improve the current treatment methods used to treat *H. pylori* infection.

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