The Curious Case of Helicobacter Pylori Csd4

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The bacterium *Helicobacter pylori* is estimated to persistently infect about half of the world's population. While most cases are asymptomatic, *H. pylori* infection is a major risk factor for gastric ulcers and cancers. Previous work had identified genes that are important for the bacterium's helical shape and efficient murine stomach colonization, and were collectively referred to as *csd* (cell shape determinant) genes (Sycuro *et al*., 2010, 2012). The *csd* genes encode proteins with a wide array of biochemical activities, highlighting the complexity of establishing and maintaining helical morphology. One of the *csd*genes, *csd4*, was found to encode a carboxypeptidase that trims non-cross-linked peptides in the peptidoglycan layer, a key structural scaffold in bacterial cells. Deletion of *csd4* resulted in straight or slightly curved rods with impaired stomach colonization in murine models of H. pylori infection (Sycuro *et al*., 2012). However, it has remained unclear how Csd4 regulates helical shape. A new Fred Hutch study led by graduate student Kris Blair in the lab of Dr. Nina Salama (Human Biology Division) and collaborators from the University of British Columbia, solved the structure of Csd4 in order to elucidate its mechanism of action. Their results were recently published in the *Journal of Biological Chemistry*.

The 1.40 Å resolution crystal structure revealed an N-terminal carboxypeptidase domain (pink in panel B of figure), followed by two smaller domains of unknown function that were found to be required for stable Csd4 expression (blue and yellow in panel B of figure). Since the initial crystal structure did not contain bound zinc in the active site, the authors soaked Csd4 crystals in zinc, which resulted in changes in active site ligands. Another crystal solved to 1.75 Å resolution in the presence of a tripeptide substrate, maintained the overall fold of the zinc-only crystal. Next, the investigators examined the role of glutamine 46 (Q46) in Csd4's catalytic activity by constructing three active site variants (Q46H, Q46A and Q46E) and found that all three variants had impaired enzymatic activity, though the Q46H mutant retained about 50% activity in some buffer conditions. To probe the role of Q46 in normal helical shape, the authors generated H. pylori strains expressing either Q46H or Q46A at the native locus and discovered that neither one of the mutant proteins were able to rescue the helical shape defect of a *csd4* loss-of-function mutant, even when present in two copies. Finally, the researchers created a strain expressing both a wild-type *csd4* and a *csd4Q46H* allele and observed a dominant-negative effect, meaning that the CsdQ46H protein prevented the wild-type Csd4 from performing its normal function. Intriguingly, the Q46H allele still

increased cell length, suggesting that not all of Csd4's functions require its enzymatic activity.

"From our perspective this paper is interesting for a number of reasons. First, the crystal structure revealed two C-terminal domains of unknown function that we found to be required for stable Csd4 expression. Second, and as the title of the paper implies, the active site of *H. pylori* Csd4 contains an atypical glutamine in place of a generally conserved histidine found in related carboxypeptidases. Much to our surprise, substitution with the histidine was insufficient to restore protein function *in vivo*. Overexpression of the Csd4-Q46H variant was unable to rescue helical cell shape but led to an increase in cell-body axis length, indicating a possible role for domains 2 and 3 in cell elongation. Co-expression of both variants in the same cell resulted in non-helical bacteria suggesting cooperativity of Csd4 protein as part of a protein complex. Curiously, this conserved site seems to be toggling back and forth between the two residues in the Epsilonproteobacteria, but does not appear to track with the cell shape of the organism in which it is found," summarized Mr. Blair.

Overall, this study presented mechanistic insight into how Csd4 functions by solving its substrate-bound crystal structure and by demonstrating that glutamine, an uncommon zinc ligand, is essential for enzymatic activity and for the normal helical shape of H. pylori. It also suggests that Csd4 is a founding member of a new family of carboxypeptidades.

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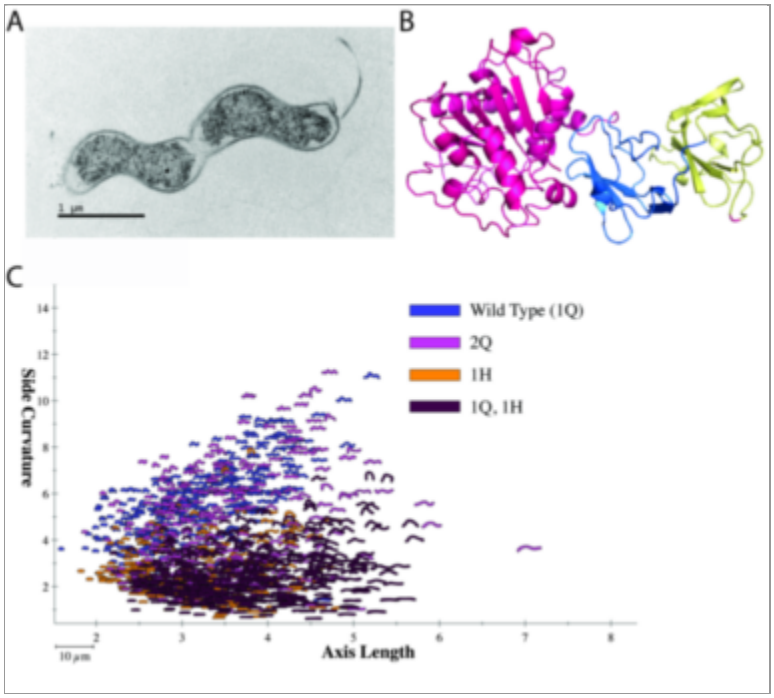

Image provided by Mr. Blair, Ms. Jenny Taylor and Scientific Imaging

A. Transmission electron micrograph of wild-type Helicobacter pylori. B. Crystal structure of Csd4 showing three-domain architecture (catalytic domain is pink and c-terminal domains of unknown function are blue and yellow). C. Scatter plot of cell side-curvature vs. cell axis-length of wild-type and mutant h. pylori bacteria (>200 cells/plot). Strain labels indicate the copy number (1 or 2) and amino acid residue at position 46 (1 or 2).