# Micro**Commentary**

# Form equals function? Bacterial shape and its consequences for pathogenesis

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## Summary

**Bacteria exhibit a wide variety of morphologies. This could simply be a consequence of an elaboration of bacterial cellular architecture akin to the famous decorative but not structurally essential Spandrels in the Basilica di San Marco in Venice that are a side-effect of an adaptation, rather than a direct product of natural selection. However, it is more likely that particular morphologies facilitate a specific function in cellular physiology. Two recent publications including one in this issue of *Molecular Microbiology* and another in *Cell* provide new insights into the molecular basis for the helical shape of the bacterium *Helicobacter pylori* and the role of this shape in pathogenesis. They identify a novel endopeptidase that is necessary to generate the helical shape by processing the peptidoglycan and report that catalytically inactive mutants lead to defects in colonization that appear to be independent of an effect on cellular motility. Here, we put these findings in the context of some of what is known about peptidoglycan and cell shape and suggest that the role of this endopeptidase in forming coccoid morphology may be critical for pathogenesis.**

An unbelievably great company of living animalcules, a-swimming more nimbly than any I had ever seen up to this time. The biggest sort . . . bent their body into curves in going forwards . . .

*Antonie van Leeuwenhoek*, Letter to Royal Society, 17 September 1683

In what was an early, if not the earliest, observation of bacteria, Antonie van Leeuwenhoek described bacteria with a curved, helical-like shape. Two key questions relevant to the papers from the Boneca (Bonis *et al.*, 2010) and Salama laboratories (Sycuro *et al.*, 2010) emerge from this prescient report. First, what is the mechanism responsible for the regulation of the rod shape and deviation from the rod shape respectively? And, second, what are the implications of this shape for cellular physiology, and in particular, for pathogenesis? The latter question has taken on particular importance with the realization in the last 25 years that a bacterium with helical shape, *Helicobacter pylori*, successfully colonizes the stomachs of ~50% of the world population and is closely associated with the development of gastric cancers and ulcers in some of those individuals (Cover and Blaser, 2009).

Bacterial shape is largely determined by peptidoglycan, the major component of the cell wall. The isolated sacculus comprising the peptidoglycan molecule that remains following gentle shearing and treatment with SDS to remove the cytosol and lipids retains the cell shape. Peptidoglycan consists of repeated glycan strands composed of $\beta$-(1,4) linkages between *N*-acetylglucosamine (NAG) and *N*-acetylmuramic acid (NAM) sugars that have covalent cross-links between NAM-associated peptides (Vollmer *et al.*, 2008). In *Escherichia coli*, cryoelectronmicroscopy reveals a single layer of glycans parallel to the cell surface oriented perpendicular to the long axis of the cell (Gan *et al.*, 2008). The peptidoglycan polymer forms a layer of varying thickness surrounding the inner membrane with Gram-positive organisms being much thicker [e.g. *Bacillus subtilis* = ~30 nM (Hayhurst *et al.*, 2008)] as compared with Gram-negative organisms [e.g. *E. coli* = ~6 nm and *Pseudomonas aeruginosa* = ~2.5 nm (Matias *et al.*, 2003)] (Vollmer and Seligman, 2010).

One key question of particular relevance to understanding the origins of the helical architecture of *H. pylori* is the precise changes to the peptidoglycan that result in particular morphologies. Computational modelling has shown that cell curvature in *E. coli* can be produced by decreasing the number of peptide cross-bridges along one side of the cell cylinder (Huang *et al.*, 2008) and mutations that

modify cross-linking affect shape, although the apparent redundancy of such proteins in *E. coli* makes attribution to a specific protein difficult (Denome *et al.*, 1999). Spores of the Gram-positive bacterium *B. subtilis* contain a thick layer of peptidoglycan and are capable of modulating their shape to accommodate dehydration. This flexibility has been attributed to the very low cross-linking of the spore cortex peptidoglycan (Popham and Setlow, 1993). In addition, although these changes to peptidoglycan structure occur post synthesis, cell shape can also be directly affected by peptidoglycan synthesis itself, either muropeptide synthesis or polymerization. For example, during sporulation, asymmetric cell division forms two differently sized cells and, eventually, the larger cell completely surrounds and engulfs the smaller cell. This dramatic shape change from a rod-shaped cell to a spherical cell is, at least in part, the result of the production of muropeptides and their subsequent polymerization (Meyer *et al.*, 2010) and suggests that peptidoglycan synthesis itself can serve to drive shape changes. Consistent with these results, modelling indicates that the processivity of peptidoglycan polymerization can provide a sufficient mechanism to account for rod-like morphologies (Sliusarenko *et al.*, 2010).

The vibroid *Caulobacter crescentus* has a curved but not helical shape. A null mutation in the gene encoding the intermediate filament-like protein crescentin causes the normally curved cells to become straight (Ausmees *et al.*, 2003) and is connected to changes in the peptidoglycan structure – specifically, differential rates of monomer insertion – that allow curvature (Cabeen *et al.*, 2009). In fact, heterologous synthesis of crescentin in *E. coli* induces curvature, suggesting that it is sufficient to produce this alternative morphology. The cytoskeletal protein MreB interacts with the cell wall enzymes PBP1 and LytE (Carballido-Lopez *et al.*, 2006; Kawai *et al.*, 2009) and since crescentin directly interacts with MreB (Charbon *et al.*, 2009), the effects of crescentin on cell shape may be mediated by MreB. The relevance, however, of this mechanism to formation of helicity in *H. pylori* is unclear given the reported lack of phenotype associated with a null mutation in *mreB* (Waidner *et al.*, 2009).

An advantage to *Helicobacter* for studying the mechanisms responsible for cell shape is its relatively compact genome. For example, *E. coli* contains numerous PBPs (at least 12) (Sauvage *et al.*, 2008), some of which have redundant phenotypes for shape determination as uncovered by deletion analysis (Denome *et al.*, 1999). In contrast, *H. pylori* has only three PBPs: HP0597, a homologue of *E. coli* PBP1A (*ponA*); HP1556, a homologue of *E. coli* PBP3 (*ftsI*); and HP1565, a homologue of *E. coli* PBP2 (*pbpA*). Bonis *et al.* started with the observation that *H. pylori* exhibits low intrinsic carboxypeptidase activity, likely due to the absence (by BLAST-based homology searches)

of genes encoding low-molecular-weight PBPs that function as D,D-carboxy- and endo-peptidases in other bacteria [e.g. *E. coli* PBP5 (*dacB*), PBP6 (*dacA*), PBP6B (*dacC*), PBP7 (*dacD*) (Sauvage *et al.*, 2008)]. These proteins have been studied in a number of bacteria, notably *E. coli*, and are known to be involved in the maintenance of shape (Nelson and Young, 2001).

Using a genome mining strategy, Bonis *et al.* identified *hp0506*, a gene that encoded a putative peptidase. Disruption of *hp0506* led to morphological changes such as branching, where a supplementary pole was observed, that were suggestive of a defect in cell wall metabolism. This phenotype was recapitulated in strains expressing mutant forms of HP0506 with substitutions in the predicted metal binding residues. Careful comparison of the peptidoglycan in the mutant strains as compared with wild-type strain indicated an increase in the levels of the pentapeptide sugar moiety, again suggestive of a role for HP0506 in processing this substrate. Finally, using a purified His$_6$-tagged protein, they confirmed that HP0506 could release the tetrapeptide from the pentapeptide and cleave the crosslink, thus confirming that it is a D,D-carboxypeptidase and a D,D-endopeptidase. The similarities in the morphological phenotypes of the *H. pylori* HP0506 and *E. coli* PBP5 mutants are consistent with the known carboxypeptidase activity of PBP5 (Ghosh *et al.*, 2008).

Cell shape as determined by proteins such as PBP5 plays a key role in motility in a number of bacteria, including helical species (Young, 2006). The motility of *H. pylori* plays a critical role in pathogenesis, as non-motile mutants were significantly defective in colonization of gastric mucosa (Ottemann and Lowenthal, 2002). One attractive hypothesis is that the helical shape enhances motility in the relatively viscous environment of the mucosa and thereby enhances colonization. Both *H. pylori* N6 X47 *hsp0506* mutants that exhibit aberrant morphologies (Bonis *et al.*, 2010) and *H. pylori* G27 *ccd1 csd1* mutants that lack the pronounced helicity of the parent (Sycuro *et al.*, 2010) exhibited significant defects in *in vivo* models of colonization. However, both mutants had normal motility when assayed on soft agar plates. At first glance, these observations appear to throw into question the role of shape in colonization. There are two possible explanations. First, it is not clear that the hydrodynamics of bacteria moving through agar is representative of their movement in gastric mucosa and, in fact, is almost certain to be different (Lauga and Powers, 2009). One way to address these differences would be to assay motility in mucus gels derived from intestinal slices, as pioneered by Freter and colleagues 30 years ago in their seminal studies of *Vibrio cholera* (Freter *et al.*, 1981). They carefully demonstrated the requirement for both the chemotaxis and motility systems for bacterial movement under these perhaps more physiologically relevant conditions. Interestingly, as they also were examin-

ing a species exhibiting deviations from a straight, rod shape, their methodology likely would be informative with the *H. pylori* shape mutants.

Alternatively, as suggested by Bonis *et al.*, cell shape may have a more indirect effect on colonization. Some bacterial proteins are enriched at one pole or both and this distribution has important implications for cellular physiology (Dworkin, 2009). Although the cellular distribution of adhesins – molecules that mediate the direct physical interactions of bacteria and host cells – has not been systematically studied, the *H. pylori* adhesin HpaA is found preferentially at the poles (Lundstrom *et al.*, 2001). Thus, the presence of a substantial fraction of branched cells in the *hp506* mutants could lead to a reduced number of adhesins per pole and thereby reduce attachment and subsequent colonization. While *H. pylori* cells lacking HpaA are deficient in colonization (Carlsohn *et al.*, 2006), it should be possible to examine the (presumably) more subtle effect of *hp0506* on subcellular HpaA distribution and on the numerous other *H. pylori* adhesins (Alm *et al.*, 2000).

Why the lack of motility has such a pronounced effect on colonization is, however, unclear. This phenotype may be the result of a number of factors that contribute to effective colonization, including an impaired ability to locate nutrients through chemotactic strategies (Williams *et al.*, 2007) or to physically navigate the mucosal environment (Freter and Jones, 1983). However, an alternative explanation comes from the observation of Bonis *et al.* that overexpression of HP0506 induces the transition to coccoid shape. Bacilli typically transform into small spherical cells in response to starvation and other physiological stressors (Roszak and Colwell, 1987). *H. pylori* becomes coccoid upon entry into stationary phase and in response to various stresses, and a mutation in the *amiA* gene that encodes a putative peptidoglycan hydrolase impairs this transformation (Chaput *et al.*, 2006). The effect of HP0506 is similar to that observed following overexpression of *E. coli* PBP5, which results in transformation of rod-shaped cells into spherical forms (Markiewicz *et al.*, 1982). In *Vibrio parahaemolyticus*, which transforms into spheres when subjected to starvation (Jiang and Chai, 1996), concomitant changes were observed in the spatial organization of MreB (Chiu *et al.*, 2008). Since MreB plays a critical role in organizing peptidoglycan synthesis and modifications, these changes may have important consequences for the activity of proteins that mediate peptidoglycan modifications. Consistent with this hypothesis, computational modelling conducted in KC Huang's laboratory suggests that changes in peptidoglycan processing can directly affect cell shape (Fig. 1; L. Furchgott and K.C. Huang, unpubl. data).

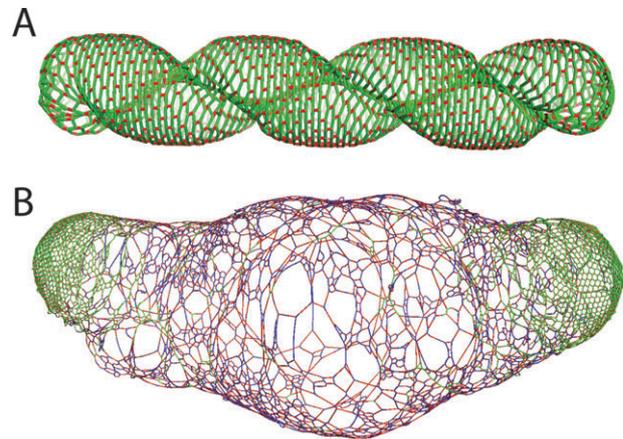Interestingly, levels of the *E. coli* carboxypeptidase PBP6 are much higher (~10-fold) in stationary phase as



**Fig. 1.** Transformation of rods into helical and coccoid morphologies.
A. A model cell wall in which the peptidoglycan has been altered along a helical path around an initially rod-shaped cell wall to produce a helical shape.
B. Preferential addition of new material near midcell, along with cleavage of old glycan strands, results in swelling of a rod-shaped model cell wall into a more coccoid shape.
Simulations were conducted by Leon Furchtgott in the Huang laboratory (Stanford) and the figure was kindly provided by K.C. Huang.

compared with growing cells (Buchanan and Sowell, 1982) and it has been suggested that PBP6 plays a key role in stabilization of the peptidoglycan during stationary phase (van der Linden *et al.*, 1992). Given the enzymatic similarities and the mutant morphologies, HP0506 could play a similar role in *H. pylori* and thereby indirectly facilitate colonization by transforming the cells into morphotypes that are better able to survive in the host.

## Acknowledgements

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