Published in final edited form as:

Mol Microbiol. 2010 November ; 78(4): 792–795.

Form equals function? Bacterial shape and its consequences for pathogenesis

Jonathan Dworkin

Department of Microbiology and Immunology, College of Physicians and Surgeons, Columbia University, New York, NY 10032 USA

Abstract

Bacteria exhibit a wide variety of morphologies (Young, 2006). 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 (Gould & Lewontin, 1979). 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* (Bonis *et al.*, 2010) and another in *Cell* (Sycuro *et al.*, 2010) 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,
September 17, 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 & 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

*701 W. 168th St., Room 1218, New York, NY 10032. T: 212.342.3731, F: 212.305.1468., jonathan.dworkin@columbia.edu.

shape. Peptidoglycan consists of repeated glycan strands composed of β -(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 *E. 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., *B. subtilis* = ~30 nm (Hayhurst *et al.*, 2008)) as compared to Gram-negative organisms (e.g., *E. coli* = ~6 nm and *Pseudomonas aeruginosa* = ~2.5 nm (Matias *et al.*, 2003)) (Vollmer & 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 modeling has shown that cell curvature in *E. coli* can be produced by decreasing the number of peptide crossbridges along one side of the cell cylinder (Huang *et al.*, 2008) and mutations that modify crosslinking 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 *Bacillus 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 & 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 mucopeptide synthesis or polymerization. For example, during sporulation of the Gram-positive bacterium *Bacillus subtilis*, 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 mucopeptides and their subsequent polymerization (Meyer *et al.*, 2010) and suggests that peptidoglycan synthesis itself can serve to drive shape changes. Consistent with these results, modeling 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). By contrast, *H. pylori* has only three PBPs: HP0597, a homolog of *E. coli* PBP1A (*ponA*); HP1556, a homolog of *E. coli* PBP3 (*ftsI*); and HP1565, a homolog 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 to 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 His₆-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 & 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 *ccdI csdI* 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. However, 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 & 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 examining 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 & 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 & 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 & 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 modeling conducted in KC Huang's laboratory suggests that changes in peptidoglycan processing can directly affect cell shape (Fig. 1; Furchgott and Huang, unpublished data).

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

Acknowledgments

I thank KC Huang for comments and for providing the images shown in Fig. 1. Work in my laboratory on peptidoglycan is supported by NIH GM081238.

References

- Alm RA, Bina J, Andrews BM, Doig P, Hancock RE, Trust TJ. Comparative genomics of *Helicobacter pylori*: analysis of the outer membrane protein families. *Infect Immun.* 2000; 68:4155–4168. [PubMed: 10858232]
- Ausmees N, Kuhn JR, Jacobs-Wagner C. The bacterial cytoskeleton: an intermediate filament-like function in cell shape. *Cell.* 2003; 115:705–713. [PubMed: 14675535]
- Bonis M, Ecobichon C, Guadagnini S, Prevost MC, Boneca IG. A M23B-family metallopeptidase of *Helicobacter pylori* required for cell shape, pole formation and virulence. *Mol Microbiol.* 2010 (this issue).
- Buchanan CE, Sowell MO. Synthesis of penicillin-binding protein 6 by stationary-phase *Escherichia coli*. *J Bacteriol.* 1982; 151:491–494. [PubMed: 7045084]
- Cabeen MT, Charbon G, Vollmer W, Born P, Ausmees N, Weibel DB, Jacobs-Wagner C. Bacterial cell curvature through mechanical control of cell growth. *EMBO J.* 2009; 28:1208–1219. [PubMed: 19279668]
- Carballido-Lopez R, Formstone A, Li Y, Ehrlich SD, Noirot P, Errington J. Actin homolog MreBH governs cell morphogenesis by localization of the cell wall hydrolase LytE. *Dev Cell.* 2006; 11:399–409. [PubMed: 16950129]
- Carlsohn E, Nystrom J, Bolin I, Nilsson CL, Svennerholm AM. HpaA is essential for *Helicobacter pylori* colonization in mice. *Infect Immun.* 2006; 74:920–926. [PubMed: 16428735]
- Chaput C, Ecobichon C, Cayet N, Girardin SE, Werts C, Guadagnini S, Prevost MC, Mengin-Lecreulx D, Labigne A, Boneca IG. Role of AmiA in the morphological transition of *Helicobacter pylori* and in immune escape. *PLoS Pathog.* 2006; 2:e97. [PubMed: 17002496]

- Charbon G, Cabeen MT, Jacobs-Wagner C. Bacterial intermediate filaments: in vivo assembly, organization, and dynamics of crescentin. *Genes Dev.* 2009; 23:1131–1144. [PubMed: 19417107]
- Chiu SW, Chen SY, Wong HC. Localization and expression of MreB in *Vibrio parahaemolyticus* under different stresses. *Appl Environ Microbiol.* 2008; 74:7016–7022. [PubMed: 18820055]
- Cover TL, Blaser MJ. *Helicobacter pylori* in health and disease. *Gastroenterology.* 2009; 136:1863–1873. [PubMed: 19457415]
- Denome SA, Elf PK, Henderson TA, Nelson DE, Young KD. *Escherichia coli* mutants lacking all possible combinations of eight penicillin binding proteins: viability, characteristics, and implications for peptidoglycan synthesis. *J Bacteriol.* 1999; 181:3981–3993. [PubMed: 10383966]
- Dworkin J. Cellular polarity in prokaryotic organisms. *Cold Spring Harb Perspect Biol.* 2009; 1:a003368. [PubMed: 20457568]
- Freter R, Allweiss B, O'Brien PC, Halstead SA, Macsai MS. Role of chemotaxis in the association of motile bacteria with intestinal mucosa: *in vitro* studies. *Infect Immun.* 1981; 34:241–249. [PubMed: 7298186]
- Freter R, Jones GW. Models for studying the role of bacterial attachment in virulence and pathogenesis. *Rev Infect Dis.* 1983; 5(Suppl 4):S647–658. [PubMed: 6356286]
- Gan L, Chen S, Jensen GJ. Molecular organization of Gram-negative peptidoglycan. *Proc Natl Acad Sci U S A.* 2008; 105:18953–18957. [PubMed: 19033194]
- Ghosh AS, Chowdhury C, Nelson DE. Physiological functions of D-alanine carboxypeptidases in *Escherichia coli*. *Trends Microbiol.* 2008; 16:309–317. [PubMed: 18539032]
- Gould SJ, Lewontin RC. The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proc R Soc London B Biol Sci.* 1979; 205:581–598. [PubMed: 42062]
- Hayhurst EJ, Kailas L, Hobbs JK, Foster SJ. Cell wall peptidoglycan architecture in *Bacillus subtilis*. *Proc Natl Acad Sci U S A.* 2008; 105:14603–14608. [PubMed: 18784364]
- Huang KC, Mukhopadhyay R, Wen B, Gitai Z, Wingreen NS. Cell shape and cell-wall organization in Gram-negative bacteria. *Proc Natl Acad Sci U S A.* 2008; 105:19282–19287. [PubMed: 19050072]
- Jiang X, Chai TJ. Survival of *Vibrio parahaemolyticus* at low temperatures under starvation conditions and subsequent resuscitation of viable, nonculturable cells. *Appl Environ Microbiol.* 1996; 62:1300–1305. [PubMed: 8919790]
- Kawai Y, Daniel RA, Errington J. Regulation of cell wall morphogenesis in *Bacillus subtilis* by recruitment of PBP1 to the MreB helix. *Mol Microbiol.* 2009; 71:1131–1144. [PubMed: 19192185]
- Lauga E, Powers TR. The hydrodynamics of swimming microorganisms. *Rep Prog Phys.* 2009; 72:096601.
- Lundstrom AM, Blom K, Sundaeus V, Bolin I. HpaA shows variable surface localization but the gene expression is similar in different *Helicobacter pylori* strains. *Microb Pathog.* 2001; 31:243–253. [PubMed: 11710844]
- Markiewicz Z, Broome-Smith JK, Schwarz U, Spratt BG. Spherical *E. coli* due to elevated levels of D-alanine carboxypeptidase. *Nature.* 1982; 297:702–704. [PubMed: 7045683]
- Matias VR, Al-Amoudi A, Dubochet J, Beveridge TJ. Cryo-transmission electron microscopy of frozen-hydrated sections of *Escherichia coli* and *Pseudomonas aeruginosa*. *J Bacteriol.* 2003; 185:6112–6118. [PubMed: 14526023]
- Meyer P, Gutierrez J, Pogliano K, Dworkin J. Cell wall synthesis is necessary for membrane dynamics during sporulation of *Bacillus subtilis*. *Mol Microbiol.* 2010; 76:956–970. [PubMed: 20444098]
- Nelson DE, Young KD. Contributions of PBP 5 and DD-carboxypeptidase penicillin binding proteins to maintenance of cell shape in *Escherichia coli*. *J Bacteriol.* 2001; 183:3055–3064. [PubMed: 11325933]
- Ottemann KM, Lowenthal AC. *Helicobacter pylori* uses motility for initial colonization and to attain robust infection. *Infect Immun.* 2002; 70:1984–1990. [PubMed: 11895962]
- Popham DL, Setlow P. The cortical peptidoglycan from spores of *Bacillus megaterium* and *Bacillus subtilis* is not highly cross-linked. *J Bacteriol.* 1993; 175:2767–2769. [PubMed: 8478339]

- Roszak DB, Colwell RR. Survival strategies of bacteria in the natural environment. *Microbiol Rev.* 1987; 51:365–379. [PubMed: 3312987]
- Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol Rev.* 2008; 32:234–258. [PubMed: 18266856]
- Sliusarenko O, Cabeen MT, Wolgemuth CW, Jacobs-Wagner C, Emonet T. Processivity of peptidoglycan synthesis provides a built-in mechanism for the robustness of straight-rod cell morphology. *Proc Natl Acad Sci U S A.* 2010; 107:10086–10091. [PubMed: 20479277]
- Sycuro LK, Pincus Z, Gutierrez KD, Biboy J, Stern CA, Vollmer W, Salama NR. Peptidoglycan crosslinking relaxation promotes *Helicobacter pylori*'s helical shape and stomach colonization. *Cell.* 2010; 141:822–833. [PubMed: 20510929]
- van der Linden MP, de Haan L, Hoyer MA, Keck W. Possible role of *Escherichia coli* penicillin-binding protein 6 in stabilization of stationary-phase peptidoglycan. *J Bacteriol.* 1992; 174:7572–7578. [PubMed: 1447130]
- Vollmer W, Blanot D, de Pedro MA. Peptidoglycan structure and architecture. *FEMS Microbiol Rev.* 2008; 32:149–167. [PubMed: 18194336]
- Vollmer W, Seligman SJ. Architecture of peptidoglycan: more data and more models. *Trends Microbiol.* 2010; 18:59–66. [PubMed: 20060721]
- Waidner B, Specht M, Dempwolff F, Haeberer K, Schaetzle S, Speth V, Kist M, Graumann PL. A novel system of cytoskeletal elements in the human pathogen *Helicobacter pylori*. *PLoS Pathog.* 2009; 5:e1000669. [PubMed: 19936218]
- Williams SM, Chen YT, Andermann TM, Carter JE, McGee DJ, Ottemann KM. *Helicobacter pylori* chemotaxis modulates inflammation and bacterium-gastric epithelium interactions in infected mice. *Infect Immun.* 2007; 75:3747–3757. [PubMed: 17517875]
- Young KD. The selective value of bacterial shape. *Microbiol Mol Biol Rev.* 2006; 70:660–703. [PubMed: 16959965]

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 KC Huang.