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Bend into shape

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Bacteria come in a variety of shapes, as most species elaborate on the 'default' sphere to resemble ovoids, rods, bend rods, spirals, branched filaments or other more complicated forms. How cells that are under considerable turgor pressure maintain a nonspherical shape is unclear, though it is known to depend on structural elements on either side of the cytoplasmic membrane: the murein (peptidoglycan) sacculus on the outside, and forerunners of the eukaryotic cytoskeleton on the inside. In this issue, the results by Cabeen *et al* argue for one attractive mechanism whereby these elements cooperate to drive cellular morphogenesis.

The murein sacculus is one large mesh-like molecule of linear glycan strands that are covalently linked by short peptides. It surrounds the entire cytoplasmic membrane where turgor pressure stretches it considerably, primarily at the peptide cross-links. It is critical in maintaining cell shape and integrity, and its destruction (e.g. by lysozyme) causes cells to quickly convert to fragile spheres that will burst under common (hypotonic) conditions. Purified sacculi typically retain the particular shape of the bacterium it was isolated from, implying that it is somehow welded into the murein meshwork. This is likely accomplished, at least partly, by controlling where and when new murein strands are incorporated as cells elongate and divide. New strands are incorporated by the combined actions of murein hydrolases and synthases, many of which are direct targets of β-lactam antibiotics. The hydrolases break bonds in the sacculus, whereas the synthases assemble and incorporate fresh glycan strands into the 'gap' left by the hydrolases. Several, perhaps all, murein hydrolases and synthases are part of larger transmembrane murein holoenzymes. This likely ensures tight coordination between their activities, which is needed to prevent cell rupture (Cabeen and Jacobs-Wagner, 2007; den Blaauwen *et al*, 2008; Vollmer and Bertsche, 2008).

Much evidence indicates that cytoskeletal filaments on the cytoplasmic face of the membrane exert spatio-temporal control on growth and shape of the sacculus by serving as tracks for the murein (holo) enzymes. The best conserved track is laid by FtsZ, which orchestrates cytokinesis (cell fission, septation, constriction) in almost all bacteria. This forerunner of tubulin forms a ring at the prospective constriction site and then attracts and guides the murein enzymes that produce and process septal murein during the constriction process. Most nonspherical bacteria also produce one or more forms of bacterial actin (MreB), which is required to maintain nonspherical shape and usually assembles in spiral-like configurations along the long axis of the cell. Similar to the FtsZ-ring during cell constriction, MreB spirals are thought to act as tracks for murein enzymes that incorporate new murein in a spiral-like fashion during cell elongation (Cabeen and Jacobs-Wagner, 2007; den Blaauwen *et al*, 2008; Vollmer and Bertsche, 2008).

Cabeen *et al* now make the case for an entirely different mechanism whereby a third cytoskeletal element helps to control cell shape in *Caulobacter crescentus* whose name reflects its curved-rod morphology (Cabeen *et al*, 2009). Earlier, the group of Jacobs-Wagner identified a straight-rod mutant of *C. crescentus* that was defective in crescentin (CreS) (Ausmees *et al*, 2003). This 50-kD protein resembles metazoan intermediate filaments (IFs), and readily polymerizes *in vitro*. *In vivo*, CreS forms a lateral filamentous structure that invariably lines the concave side (inner curvature) of the cell, suggesting that it helps to induce cell curvature quite directly (Ausmees *et al*, 2003). Additional IF-like proteins have since been identified in other bacteria (Bagchi *et al*, 2008), suggesting that, like tubulin and actin, IFs were a prokaryotic invention as well.

Four laboratories joined forces to elucidate how CreS causes rod-shaped cells to curve (Cabeen *et al*, 2009). Remarkably, treatment of *C. crescentus* cells with the β-lactam mecillinam induced a gradual detachment of CreS from the cell periphery to yield a single filamentous structure that, once free in the cytoplasm, coiled-up with a pronounced left-handed twist. This result indicates that (1) the cellular CreS filament likely consists of some stable super arrangement (e.g. bundles) of the ~10-nm wide 'proto-filaments' that are seen *in vitro* (Ausmees *et al*, 2003), (2) association of the filament with the membrane is somehow dependent on the integrity of the murein sacculus and (3) the attached filament is normally in a stretched conformation. The latter raised the possibility that a tensed CreS filament bends a cell simply by exerting a sufficiently large compressive force to 'scrunch-up' the murein mesh-work on its side of the cylinder. However, this is inconsistent with the fact that de-proteinized *C. crescentus* sacculi retain the curved appearance of cells (Poindexter and Hagenzieker, 1982), and the authors formally show that curved cells require an extended period of growth (i.e. new murein synthesis) to straighten-out in the absence of a functional CreS structure. Rather, insertion of

new murein in curved cells seems to occur at a slightly higher rate on the convex than on the CreS-decorated concave side of the cell cylinder (Cabeen *et al*, 2009).

Hence, the authors propose a model wherein the CreS filament anisotropically biases the kinetics of new murein insertion to produce cell curvature. The model borrows from the surface-stress theory (Koch, 1983) in that turgor-induced stretching of the peptide cross-bridges of the sacculus is reasonably assumed to lower the energy barrier of their breakage, and the subsequent insertion of new strands, by murein enzyme complexes. The model also still relies on the CreS filament exerting some compressive force on the murein wall, but only to the extent that turgor-induced stretch is relieved sufficiently to slightly bias murein hydrolase/synthase activities away from the CreS side of the cylinder wall. Thus, rather than directing the location of murein holoenzymes, through which the MreB and FtsZ cytoskeletons are thought to direct cell morphogenesis, CreS is likely to do so by mechanically 'holding' their substrate in a conformation that is a bit less favourable for insertion of new material (Cabeen *et al*, 2009).

One critical question is how the CreS filament connects to the murein layer across the membrane. Strikingly, the authors find that CreS also forms a lateral filament in *Escherichia coli*, and that this causes this normally straight rod to grow curved as well. In addition, a small basic domain at the N terminus of CreS is required for lateral association of the filament with the cylinder wall, and for curved growth, in both organisms. Thus, this domain is likely required for interaction with whatever transmembrane molecules connect CreS to the sacculus, and these intermediary molecules are apparently not specific to *Caulobacter* (Cabeen *et al*, 2009). The authors briefly mention a requirement for MreB, which may implicate one or more of the known transmembrane components of the spiral MreB cytoskeleton (Gerdes, 2009) in connecting CreS to the sacculus. Solving this part of the puzzle will be illuminating.

As any good study, the work provokes thought. Is the idea that the MreB spiral and FtsZ ring merely guide the location of murein enzymes perhaps a bit too simple (or even wrong in the case of MreB)? It seems entirely possible that these cytoskeletal filaments also affect the activity of murein enzymes either by acting on them directly or, similar to what is proposed here for CreS, by physically inducing local scrunching or stretching of their sacculus substrate. Obviously, more work is needed to firmly grasp bacterial morphogenesis, but the study by Cabeen *et al* illustrates that tantalizing progress is being made.

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