

## The mechanics of shape in prokaryotes

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## 1. ABSTRACT

Bacteria derive and maintain a variety of shapes that carry selective benefits. The shapes are usually defined by a mechanically stiff exoskeletal cell wall -- a macromolecular network of peptidoglycan. The growth of such a network is catalyzed by transglycosylases and transpeptidases, and various cell-wall remodeling enzymes further digest and process the network. To maintain the overall cell shape, the bacterial cytoskeleton coordinates cell wall synthesis on the cellular scale. Recent studies also suggest that the mechanical properties of the bacterial cytoskeleton are important for cell wall growth. Here, we review current experiments and theories on the structure, dynamics and interactions of the bacterial cell wall and cytoskeleton, and their contributions to cell shape maintenance. We also propose future research directions that will help clarify the mystery of bacterial cell morphogenesis.

## 2. INTRODUCTION

Self-reproduction is one of the fundamental characteristics of life. In all life forms, self-reproduction always involves the syntheses of biopolymers with high structural fidelity. In the central dogma of biology, for example, DNA replication, RNA transcription and protein translation are basic polymer-synthesis events, during which the loss of fidelity may lead to mutations or lethality. A common feature among the three processes is that they all initiate from a one-dimensional template. Given the linear structures of DNA and mRNA, polymer synthases (DNA polymerase, RNA polymerase or ribosome) can simply “walk” along them, and assemble the new polymer according to the encoded genetic information, although the resultant polymer may fold into two- or three-dimensional structures afterwards.

For most bacteria, another crucial biopolymer to replicate is the cell wall. This structure defines a beneficial

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cell shape, confines the cell membrane so that bacteria do not explode due to osmotic pressure, and protects the cell from external mechanical perturbations. In comparison to the polymers above, cell wall replication and growth faces the distinct challenge that the template is already a three-dimensional network (1). Microscopic synthase molecules need to “weave” new portions of material into this network, doubling its size, and then divide it in half, all while maintaining the overall shape of the network on a much larger length-scale. Besides its shape, the new network must also maintain the cell’s mechanical integrity to carry on its function. Large weak spots or holes in the network can lead to cell lysis. Up to now, although genetic and biochemical studies have identified multiple bacterial cell wall synthases in various species (1-3), how they coordinate with each other in space and time so that the cell shape and mechanical properties can be maintained is poorly understood.

In addition to the exoskeletal cell wall, bacteria have been shown to possess an intracellular cytoskeleton (4-6). Interestingly, some cytoskeletal components are clearly involved in the spatiotemporal coordination of the cell wall synthesis, as genetic or antibiotic perturbations to these components alter the cell shape in a growth-dependent manner (4, 7-9). Early studies showed that the bacterial cytoskeleton controls cell shape by positioning the cell wall synthases (4). A recently emerging theme is that the mechanics of the cytoskeleton may also affect cell shape through the application of forces to the new cell wall material before its insertion into the existent cell wall (10), or through changes in the local cell wall insertion probability by introducing extra elastic energy (9). A detailed mechanism describing how the cytoskeleton controls cell shape remains unclear.

This review aims to summarize the current understanding of the bacterial cell wall and cytoskeleton in the determination of cell shape through a physical lens. We also discuss several future directions for the field and propose experimental and theoretical efforts along these directions.

### 3. BACTERIAL CELL SHAPE AND GROWTH

Bacteria come in a variety of shapes: Cocci (e.g. *Streptococcus pneumoniae*, *Staphylococcus aureus*) are generally spherical or spheroid shaped; Bacilli (e.g. *Bacillus subtilis*, *Escherichia coli*) are rod shaped with hemispherical end-caps, while spirochetes (e.g. *Spiroplasma melliferum*) are named for their spiral shapes (11). The more rare shapes include: curves (e.g. *Vibrio cholerae*), curves with stalk appendages (e.g. *Caulobacter crescentus*), stars (e.g. *Ancalomicrobium* species), and rod branches (e.g. *Nocardia opaca*) (12). Individual cells also sometimes form clusters (e.g. *Staphylococcus* species), chains (e.g. *Streptococcus* species), or even thick network of hyphae (e.g. *Streptomyces coelicolor*) after multiple cell divisions, further adding to the variety and complexity of cell shapes (12-13).

The various bacterial shapes carry selective benefits. For example, motile (free-swimming) bacteria usually have rod-like shapes and are less likely to be

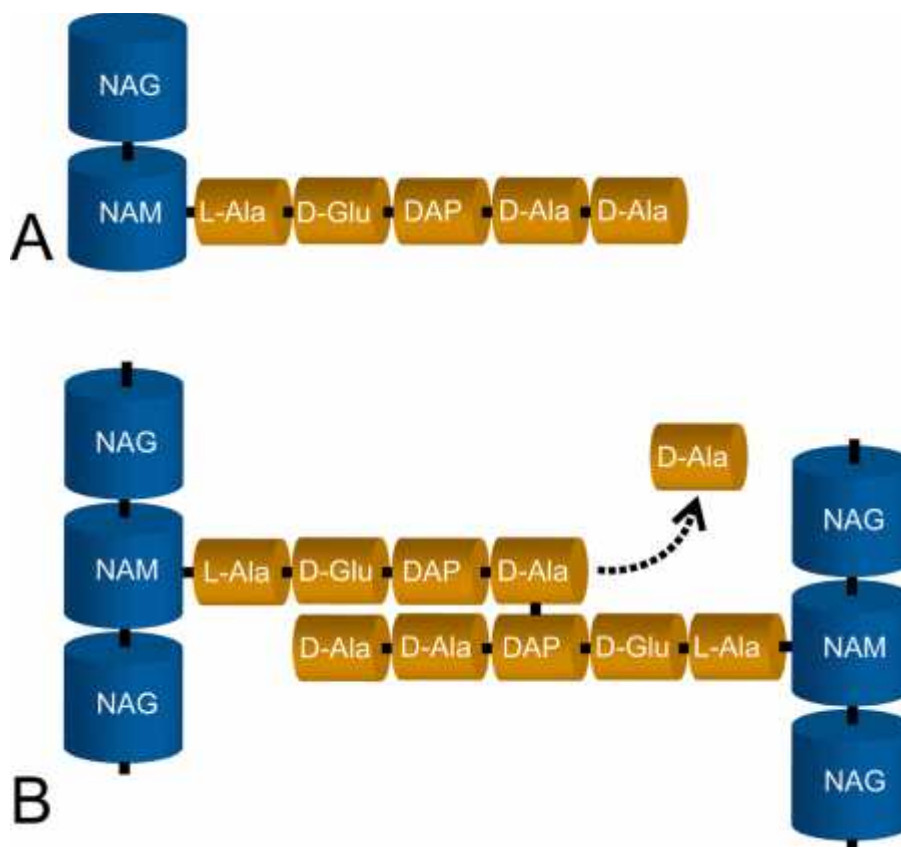
spherical than non-motile bacteria are (14). Studies have shown that a rod shape increases swimming efficiency and dramatically facilitates the temporal sensing of chemical gradients, a common mechanism of chemotaxis among motile bacteria (14-15). For a non-motile rod-shaped, aquatic bacterium, the rod shape reduces sinking speed and may help to keep a cell afloat (14).

Besides swimming and floating, cell shape may also be beneficial to those bacteria which colonize and form biofilms. A study observing cells colonizing in a small chamber indicated that the rod shape helps cells to self-organized into an ordered super-structure that increases nutrient access and waste evacuation efficiencies (16). Other putative benefits of the rod shape include the increased surface area, which may be advantageous in nutrient-limited conditions; and increased ability to produce substrate attachment because of a rod’s resistance to fluid shear (12).

Although some bacteria have flexible cell shapes, such as *Bdellovibrio bacteriovorus* which can have either nearly-straight rod or highly bent conformations (17), the general shape of most species is preserved through multiple rounds of cell proliferation. For instance, rod species *Escherichia coli* and *Bacillus subtilis* proliferate through elongational growth and mid-cell division, the former of which especially ensures the propagation of the rod shape. During elongational growth, the cell length increases while the cell radius is maintained. Such a feature is highly non-trivial, as computer simulations suggest that given a uniform osmotic pressure, the cell radius would also increase during growth (10). How rod-shaped bacteria manage to preserve their radii demands more mechanistic studies.

### 4. BACTERIAL CELL WALL

The bacterial cell envelope consists of a phospholipid cell membrane (inner membrane) and a peptidoglycan cell wall (11). Gram-negative bacteria further have a phospholipid outer membrane, while Gram-positive species lack this structure (11). The peptidoglycan cell wall defines the static cell shape in most species. Chemically purified cell walls maintain the characteristic cell shape (11). Extracted walls, sometimes termed sacculi, are typically purified by boiling in sodium dodecyl sulfate to destroy lipid membranes and denature proteins along with a subsequent digestion of other cellular contents with DNase, RNase and -chymotrypsin. (18). In contrast, digestion of the cell wall in a rod-shaped bacterium leads to the formation of spheroplast, also called a protoplast, a wall-less spherical cell enclosed by the cell membrane (11). Atomic force microscopy (AFM) measurements on purified *E. coli* cell walls find a Young’s modulus of 25-45 MPa, suggesting that the cell wall has an elasticity comparable to that of soft rubber (18). Although the purification of the cell wall may affect its mechanical property, the number above is within the range of cell wall young’s moduli measured in other intact bacteria using either AFM or optical tweezers (1.8 MPa for *Staphylococcus aureus* (19) and 50 MPa for *Bacillus subtilis* (20)).



**Figure 1.** Bacterial cell wall structure. (A) Cell wall subunit. (B) Cross-link between glycan strands.

#### 4.1. Cell wall structure

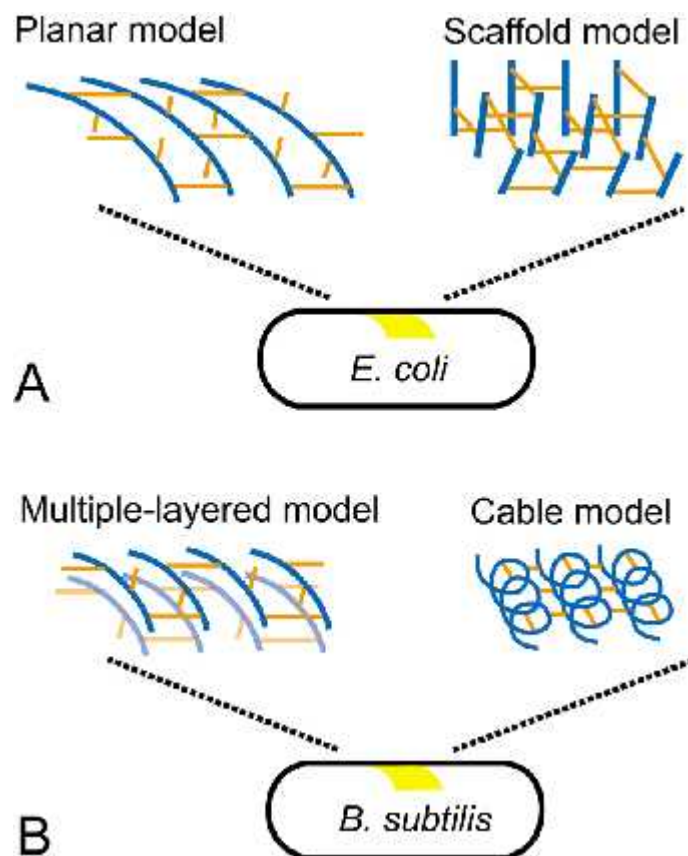
The bacterial cell wall is made from a covalently linked network of peptidoglycan (1). In the network, N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) molecules are linked by glycosidic bonds and alternates in glycan strands. Nearby strands are cross-linked through peptides stemmed from the NAM molecules. For *B. subtilis* and *E. coli*, the peptides consist of, from NAM: L-Ala, D-Glu, diaminopimelic acid (DAP), D-Ala and D-Ala (Figure 1A). The second to last D-Ala in such a pentapeptide forms a peptide bond with the DAP in another pentapeptide, while releasing the terminal D-Ala (Figure 1B), so that nearby glycan strands can be cross-linked (3).

In the Gram-negative bacterium *E. coli*, studies suggest that the peptidoglycan is a single-layered, planar network with glycan strands lying in the near circumferential direction, and peptide cross-links pointing in the near longitudinal direction (21) (Figure 2A). The evidence supporting this architecture includes: a) upon hydrolysis of the peptide links, the cell wall ruptures preferentially along the circumferential direction (22); b) sonication, which breaks peptide links more effectively than glycan strands, also leads to ruptures in the circumferential direction (23); c) AFM measurements suggest the cell wall is stiffer in the circumferential direction than in the longitudinal direction (18); d) electron cryotomography reveals glycan strands in the circumferential direction (24).

An alternative model of the *E. coli* cell wall architecture, termed the “scaffold model,” suggests that the glycan strands point in the radial direction of the cell cylinder, while peptide links lie in the plane of the cell wall (25) (Figure 2A). This model, which conflicts with many of the observations listed above, requires more peptidoglycan than exists in *E. coli* to cover the whole cell surface (21). Moreover, the model generates conflict between the measured average glycan strand length (25 to 35 NAG-NAM units, or 25 to 35 nm) and the thickness of the cell wall (at most 4-8 nm) (21, 24, 26-27). Thus, this model has become unflavored in the community.

Although the cell wall of the Gram-positive bacterium *B. subtilis* has the same chemical content as that of *E. coli*, it is much thicker (~40nm), suggesting multiple peptidoglycan layers or other complex structures (28). An AFM study further reveals 50-nm wide peptidoglycan cables in the circumferential direction with cross-cabling striations (29). The authors of this study propose that the cables are made of coiled glycan strands (Figure 2B).

Details of the structural mechanics of the peptidoglycan are crucial for the maintenance of cell shape. The peptide cross-links are thought to be relatively compliant based on computational studies of the alternating D and L amino acids (30), while the glycan strands are often considered to be more rigid given their molecular structure (31). In other computational work, Huang *et al.*



**Figure 2.** Models of bacterial cell wall architecture. (A) Single-layered planar model and scaffold model of *E. coli* cell wall. (B) Multiple-layered model and cable model of *B. subtilis* cell wall. The cartoons are only schematic. For the multiple-layered model, the number of layers is not exact. For the cable model, the number of glycan coils in each cable and the pitch of coils are not exact.

(32) modeled both the peptide cross-links and the glycan segments between adjacent cross-links as linear springs using a spring constant of  $10^{-2}$  N/m for the peptide cross-links and an elastic constant approximately 5 times stiffer for the glycan segments. These parameters yield a cell wall Young's modulus within the range of experimental measurements (18, 32), and a glycan strand persistence length that matches current estimates (32-33). In addition, these authors show that the combination of turgor pressure and the anisotropic nature of the cell wall elasticity can yield complex cell shapes such as spirals.

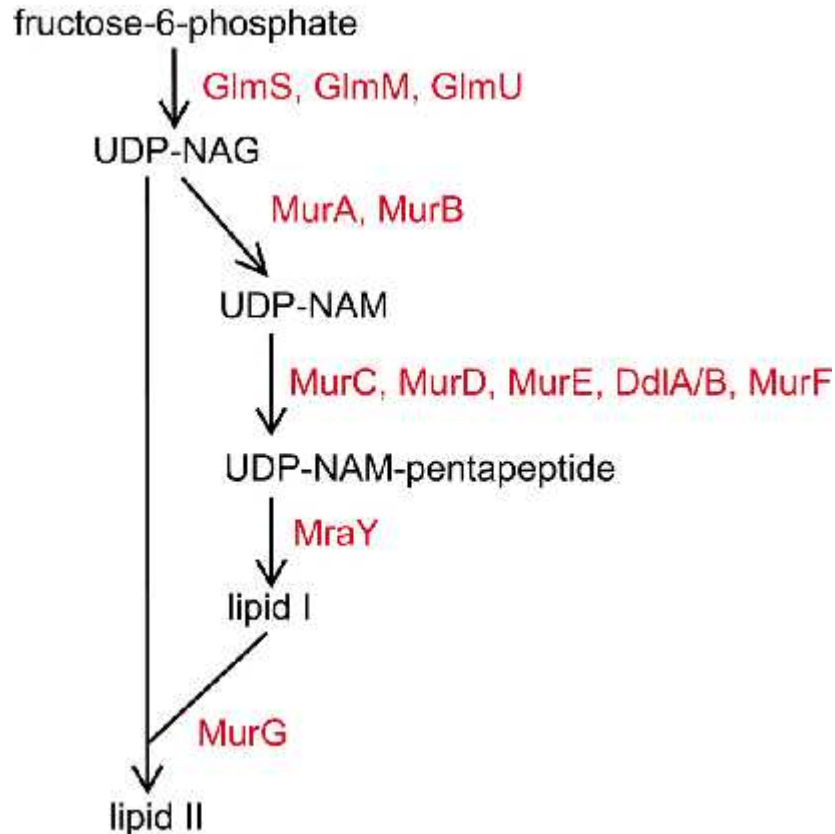
#### 4.2. Cell wall synthesis

The biochemistry of cell wall synthesis has been very well studied. First, in the cytoplasm, UDP-N-acetylglucosamine (UDP-NAG) is synthesized from fructose 6-phosphate with the help of the enzymes GlmS, GlmM and GlmU. Then MurA and MurB catalyze the synthesis of UDP-N-acetylmuramic acid (UDP-NAM) from UDP-NAG. Next, the amino acids in the pentapeptide are subsequently linked to UDP-NAM by enzymes MurC, MurD, MurE, DdlA/B and MurF. MraY then transfers UDP-NAM-pentapeptide to cell membrane acceptor bactoprenol, generating lipid I. Next, MurG adds UDP-NAG to lipid I, yielding lipid II (Figure 3). Finally, this

disaccharide subunit is flipped into the periplasm through an unknown mechanism, ready to be added to the end of a glycan strand (2).

In the periplasm, new disaccharide subunits are linked to the existent peptidoglycan network through transglycosylation and transpeptidation reactions (1). The former generates the glycosidic bonds along the glycan strands; the latter forms the peptide bonds in the cross-links. These reactions are catalyzed by high molecular weight penicillin binding proteins (HMW PBPs). In *E. coli*, transglycosylation is catalyzed by PBP1a, 1b and 1c; transpeptidation is catalyzed by PBP1a, 1b, 2 and 3. In addition, non-penicillin binding protein Mgt also catalyzes transglycosylation (3).

How different transglycosylases and transpeptidases coordinate in peptidoglycan synthesis is unknown. Among the PBPs, PBP1a and PBP1b seem to have redundant functions, as neither of these proteins is essential, but a double mutant is not viable (34). PBP1c deletion has no obvious phenotype, and PBP1c overexpression does not rescue the PBP1a/1b double mutation (35). PBP2 depletion leads to a gradual loss of the rod shape. Cells can proliferate as small spheres under



**Figure 3.** The biochemical pathway of the cell-wall precursor synthesis.

conditions of low growth rate, but form large, non-dividing spheroids at high growth rate (36). PBP3 inhibition results in a nondividing filamentous phenotype (37). The diversity in these phenotypes suggests that different PBPs function in different spatiotemporal stages and/or with different protein partners to ensure controlled cell-wall growth and division.

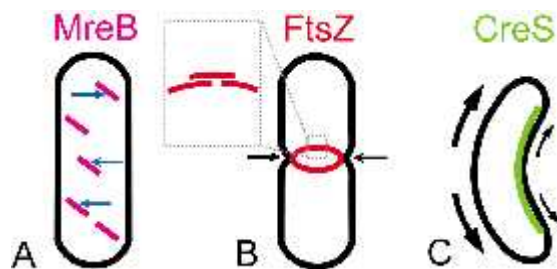
The global, spatial pattern of cell wall synthesis in *E. coli* has been difficult to pin down. It was first found to be diffuse when probed using radioactively-labeled diaminopimelic acid (DAP) (1). In these experiments, labeled, newly-synthesized wall material forms disperse foci on the cell periphery with an increased density at the division site (38). Later experiments utilized a periplasmic amino acid exchange reaction to label existing peptidoglycan with D-Cysteine. During growth, the labeled wall regions were diluted by newly-incorporated, unlabeled material. The pattern of unlabeled wall in these experiments resembled a combination of patches, bands and hoops (39-40). Further insight into the global pattern of cell wall synthesis came from labeling nascent externalized lipid II in Gram-positive cells with a fluorescent derivative of vancomycin. Such a method reveals that rod-shaped Gram-positive cells have two distinct ways to achieve elongational growth, either by inserting new material in an approximately helical pattern along the cylindrical cell wall (e.g. *B. subtilis*), or by polar growth (e.g. *Corynebacterium glutamicum*) (41).

Consistent with the helical insertion pattern observed through vancomycin labeling, some *B. subtilis* mutants form twisted and coiled macrofibers after germination (42). In these mutants, cell division is blocked and both cell poles remain attached to the spore coat. After germination, the cell midline initially grows as a circle and later starts to coil, forming a double helical or more complicated spiral structure of the cell itself. Depending on the strains and growth environment, the helix can either be left-handed or right-handed (42-45).

The distribution of newly synthesized cell wall should be determined by the localization and dynamics of active cell-wall synthesis proteins, either PBPs that directly incorporate lipid II to the existing cell wall, or upstream proteins involved in the synthesis and exportation of cell wall precursors. It is important to note that the snapshot localization of these proteins may not be able to explain the observed pattern of wall synthesis, since a processive cell-wall synthesis machinery (reviewed later) may leave “tracks” of new cell wall that themselves form the geometrically important pattern. Thus, the non-uniform distribution of new cell wall motivates time-lapse observations of the proteins involved.

#### 4.3. Cell wall remodeling

The bacterial cell wall does not remain static after being synthesized, but undergoes remodeling when necessary. In order to do so, bacteria possess a variety of



**Figure 4.** Localization, dynamics, and function of bacterial cytoskeleton. (A) Fragmented MreB filaments (magenta) move in a near-circumferential direction, powered by peptidoglycan synthesis (blue arrows). (B) FtsZ ring (red) localizing at the mid-cell consists of short protofilaments. A small constriction force from the Z-ring (black arrows) may bias the cell-wall growth inward. (C) CreS (green) filament may reduce the turgor-pressure induced strain (black arrows; thickness indicates magnitude) on one side of the cell wall, leading to less wall synthesis on that side.

native hydrolases that process specific parts of the peptidoglycan structure. For example, lytic transglycosylase cleaves the glycosidic bond between the disaccharide subunits; N-acetylglucosaminidase breaks the glycosidic bond within a disaccharide subunit; carboxipeptidase removes terminal D-Ala from the pentapeptide; amidase cuts between the peptide and the glycan strand; and endopeptidase hydrolyses peptide bonds within a crosslink (11).

Cell wall remodeling generates new sites for adding peptidoglycan subunits. Indeed, the cell wall hydrolases are often found in association with HMW PBPs (46-47), suggesting that hydrolysis and synthesis may occur in a spatiotemporally coordinated manner. In Gram-negative bacteria, old material removed from the cell wall is efficiently recycled back to the cytoplasm for future cell wall synthesis (48-49). Gram positive bacteria lack a similar recycling system and often release large amounts of peptidoglycan fragments into the surround environment. These fragments can then be used as signaling molecules including as potent germinants of dormant *B. subtilis* spores (50).

Cell wall remodeling may also affect cell shape in a growth-independent manner. Huang *et al* predicted that specific patterns of defects in a cylindrical peptidoglycan network can lead to common bacterial shapes such as curved rods, helices, and spindles (32). In support of this notion, experiments have identified peptidoglycan endopeptidases as key regulators in the helical shape of *Helicobacter pylori* (51).

## 5. BACTERIAL CYTOSKELETON

The newly discovered bacterial cytoskeleton plays crucial roles in cell growth and shape maintenance. We now know that bacteria have homologues of all three kinds of eukaryotic cytoskeletal proteins: the actin homologue MreB, the microtubule homologue FtsZ, and

the intermediate filament homologue CreS (4). Besides cell shape regulation, these and other bacterial cytoskeletal proteins are involved in chromosome and plasmid segregation, maintenance of cell polarity, assembly of intracellular organelle-like structures, and cell motility (5-6).

### 5.1. Actin homologue MreB

The actin homologue MreB serves as a key cell shape regulator in most rod bacteria (4, 52). Depletion of MreB in *E. coli* has similar effects as the depletion of Pbp2: cells grow into large, non-dividing, spheroid shapes at high growth rate, but can divide and propagate as small spheres at low growth rate (36). The morphological effect of MreB overexpression in *E. coli* depends on the growth condition as well. When grown in rich LB medium, the cells became filamentous and wider than wildtype rods, while in M9 minimal medium, cells form large spheroids and stop dividing (53).

MreB can polymerize into filaments in the presence of ATP or GTP. *In vitro*, these filaments form straight bundles, whereas *in vivo*, they assemble into a helical structure under the inner membrane (7, 54-57). MreB was shown to colocalize with the cell-wall transpeptidase PBP2 in *E. coli* (58). Consistent with a similar depletion phenotype, this suggests that MreB and PBP2 may work in the same protein complex or work next to each other to control cell shape although a direct functional connection has been difficult to ascertain. Other proteins that have been proposed to work in the same complex include MreC, MreD, RodA and RodZ (36, 58-61). It is important to note that the control of cell shape by MreB is growth-dependant. When treated with A22, an antibiotic that quickly and reversibly disassembles MreB, *E. coli* and *C. crescentus* cells do not immediately lose their shapes but gradually deform over several mass-doubling time (7-8).

In *Bacillus subtilis*, three MreB paralogs MreB, Mbl and MreBH colocalize in a right-handed helical structure and control cell morphogenesis (56, 62). Among these, Mbl was shown to determine the helical spatial patterning of new cell-wall material revealed by fluorescent vancomycin (41).

Recent observations of fluorescence-labeled MreB in *E. coli* and MreB paralogs in *B. subtilis* showed that the proteins, when expressed at endogenous or low levels, form short fragments (63-65). The extended, continuous structures observed previously (55-56) may be an overexpression or imaging artifact. The small size of the newly-observed fragments prevents the determination of its localization orientation. Thus each fragment may still be helical/diagonal on the cell membrane (Figure 4A). Most intriguingly, the fragments move persistently in a near-circumferential direction (Figure 4A), and the motion depends on cell wall synthesis (not on polymerization/depolymerization dynamics) (63-65). In *E. coli*, both the transglycosylation and transpeptidation reactions are required for the motion (63). In *B. subtilis*, the fluorescence labeled transpeptidases Pbp2A and PbpH

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showed similar motion (64-65). Together, these studies demonstrate the processive activity of the cell wall synthesis machinery *in vivo* and its coupling with MreB.

The observations above suggest that MreB controls cell shape indirectly by spatially patterning key cell-wall modulating enzymes (4). In a computational work, Furchtgott *et al.* pointed out that such function of MreB decouples cell wall insertion probability with local cell wall density, which is required to maintain the straight rod cell shape (66). Without MreB, local fluctuations in cell wall density positively feed back to local cell wall insertion probability, leading to a bent cell morphology (66). In the simulation, the authors implemented a helical MreB pattern, although other patterns that decouple the insertion probability with wall density can have the same effect (66). Indeed, the shape maintenance is not affected when the MreB helix is fragmented and each fragment is allowed to spin about the longitudinal axis of the cell (66).

Another interesting point brought up in the work of Furchtgott and coauthors is that to perfectly maintain cell radius, new glycan strands must be pre-stretched before insertion into the existent peptidoglycan, otherwise the cell radius increases (66). In a previous theoretical work that first introduced the concept of pre-stretching, Lan *et al.* attribute the pre-stretching force to the MreB, assuming MreB as a stiff, force-generating polymer (10). A separate theory explains the spiral shape of the *B. subtilis* mutants with an inextensible, helical Mbl polymer tightly linked to the cell wall (67). Although whether MreB exerts forces to the cell wall has not been tested experimentally, Wang *et al.* showed the significant contribution of MreB to *E. coli* cell rigidity *in vivo*, and verified that MreB is stiff enough to apply meaningful forces to the cell wall (68). On the other hand, prolonged forces are capable of guiding cell growth and plastically influencing cell shape. Filamentous *E. coli* cells growing in microchambers with spiral, crescent or other shapes adopt the shape of the confining chambers, and maintained the shape immediately after being released from the chambers (69). *E. coli* cells growing through microfabricated channels thinner than the cell diameter obtain a variety of anomalous shapes after exiting the channels (70). Given the success of the theories and the experiments, it is possible that MreB also plays a mechanical role in cell growth.

### 5.2. Tubulin homologue FtsZ

Tubulin homologue FtsZ functions in bacterial cell division (4). In *E. coli*, depletion of FtsZ leads to filamentous growth, while overexpression of FtsZ rescues the non-dividing phenotype of MreB-depleted cells at high growth rate (36, 55).

FtsZ polymerizes *in vitro* in the presence of GTP and catalyzes GTP hydrolysis. The polymerization dynamics and polymer structures of FtsZ has been reviewed in Ref. (71). Importantly, the FtsZ polymers have either a straight or curved conformation depending on the hydrolysis state of GTP. In particular, GTP-FtsZ favors the straight form while GDP-FtsZ tends to curve (72). Such a conformational transition has been proposed to generate constriction force during cell division (73).

*In vivo*, FtsZ assembles into a circumferential ring structure at the division site, called the “Z-ring” (Figure 4B). A closer look at the FtsZ conformation through electron cryotomography shows that the Z-ring is made of arc-like protofilaments just underneath the cell membrane (74) (Figure 4B). The filaments are ~100nm long and ~5nm in diameter, align in the near circumferential direction, and display a very regular lateral spacing (74). How FtsZ achieves this conformation is unknown.

FtsZ is one of the first proteins localized to the division septum, together with its cell-membrane anchors FtsA and ZipA (71). A modulator protein ZapA further enhances the assembly and stability of the Z-ring (71). Next, FtsZ recruits down stream proteins involved in cytokinesis, including FtsEX, FtsK, FtsQ, FtsLB, FtsW, PBP3, FtsN and AmiC, forming a complex protein structure called the divisome (75). FtsEX appear to enhance the recruitment process especially in salt-free media (76). The incorporation of FtsK, FtsQ, FtsLB, FtsW, PBP3, FtsN and AmiC occur in an interdependent order as listed (FtsQ incorporation requires FtsK; FtsLB incorporation requires FtsQ, and so on) (77-79). Among them, FtsW is hypothesized to catalyze the flipping of lipid II from the cytoplasmic side of the cell membrane to the periplasmic side (79). PBP3 is a division-specific transpeptidase as introduced previously (37). AmiC is a cell-wall amidase that removes the peptide cross-links from the glycan strands (78). Deletion of *amiC* results in a chained cell morphology, suggesting that AmiC splits the peptidoglycan septum during division (80). FtsN has weak sequence homology to amidases, thus may also function in cell-wall hydrolysis (79). However, FtsN depletion leads to filamentous growth and cell death (81), rather than cell chaining, indicating that FtsN is essential for the initiation of septum formation. Presumably, FtsN may weaken the cell wall at the division site to facilitate the constriction force from the Z-ring.

Although the exact physical mechanism of cell division is not clear, it is known that the FtsZ-induced constriction force is not sufficient to pinch and divide the cell wall or the cell membrane: An intact cell cannot divide when cell-wall synthesis at the septum is blocked (37); a wall-less spheroplast can divide only if new cell-wall synthesis is allowed (82). It has been proposed that a small constriction force from FtsZ may bias the cell-wall synthesis inward (Figure 4B), and the inward synthesis eventually leads to membrane fission and cell division (10, 83).

Intuitively cell division should be coupled with DNA replication to ensure the robust inheritance of genetic material. Indeed there is an important cell cycle checkpoint that links DNA replication, FtsZ polymerization, and the divisional synthesis of peptidoglycan. In *E. coli*, when a replication fork is stalled by DNA damage, SulA, an FtsZ-polymerization inhibitor, is produced in response (71). Structural studies suggest that SulA binds to FtsZ monomer and sequester it from the polymerization reaction (84). The inhibition of FtsZ polymerization effectively blocks cell

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division, until DNA is repaired and replication can be resumed (85).

### 5.3. Intermediate filament homologue CreS

Intermediate filament homologue CreS generates the curved conformation of *C. crescentus*. When *creS* is deleted, *C. crescentus* lose the comma-like shape and propagate as straight rods (4). CreS forms a polymer along the cell-length direction, localizes at the inner curvature of the cell (Figure 4C), and attaches to the cytoplasmic side of the cell membrane (4). Cabeen *et al.* showed that CreS filaments, when detached from the cell membrane through antibiotic treatment, collapse into a helix (9). This suggests that CreS is in a stretched form in untreated cells, and may generate a constricting force on the cell wall. The authors proposed a model that this constricting force reduces the strain on nearby peptide cross-links, and thus heightens the energy barrier of breaking them during cell wall synthesis. As a result, the side of cell wall with CreS incorporates less new material than the other side does, thus the cell develops the curved morphology (Figure 4C). Furthermore, expression of CreS in *E. coli* is sufficient to generate cell curvature (9). As the two species are distinct, the mechanism of CreS induced curvature is likely to be simply mechanical (9). It is important to note that the proposed mechanical influence is through altering cell growth rather than directly bending the peptidoglycan network, as the shape straightening after CreS disruption is slow and growth-dependent (9).

## 6. PERSPECTIVE

We predict three major trends in research into the biophysical aspects of bacterial cellular architecture and ultrastructure. First, while a list of cell wall synthesizers/remodelers have been identified, *in vivo* studies will focus on the dynamics and coordination of the cell wall synthesis machineries. Important questions along this path include: Do the various transglycosylases and transpeptidases catalyze peptidoglycan polymerization in a persistent or diffusive manner? Together with the cell wall remodelers, do they form one or multiple sets of synthesis machineries for cell growth/division? If there are several species of synthesis complexes for, e.g., rod cell elongation, do they share a common function, or have distinct tasks (e.g. one only inserts new material, while another one mainly remodels the cell wall to ensure cell shape and structural integrity)? How do they depend on bacterial cytoskeleton? Similar questions can be extended to sporulation, which includes more complicated modes of cell wall synthesis. The above questions can be answered through engineering functional fluorescence-labeled cell wall synthesizers and remodelers, and studying their dynamics and colocalization with super resolution imaging.

Second, *in vitro* studies will try to reconstitute essential enzymatic and regulatory steps of cell wall synthesis. It should be possible to track synthase motion on purified cell walls. If successful, the effects of known binding partners on the dynamics of PBPs could be tested. Indeed, the whole molecular coordination scheme may be revealed through this bottom-up approach.

Third, theoretical and *in silico* studies will predict the effects of spatial and mechanical controls of peptidoglycan synthesis on the organization and dynamics of the cell wall. For example, does the MreB-guided helical insertion result in chirality in the cell wall organization and growth dynamics? What kind of molecular mechanism can ensure the constant-radius elongation with polar growth in rod-shaped bacteria lacking an MreB homologue? And what will be the resulting peptidoglycan ordering and dynamics with the mechanism? These predictions can be tested experimentally by live cell labeling of peptidoglycan with fluorescent or physical landmarks, and observing their motion during cell growth or mechanical perturbations.

With increasing knowledge on the mechanism of bacterial cell growth and shape maintenance, we may also be able to engineer bacterial cell shape with greater facility and ease. In principle, through localizing cell wall synthesis to several restricted regions in a cell, one may generate branches, protrusions or other, more complex morphological features. Through mechanical forces applied by bacterial cytoskeleton, one might introduce constrictions, curves or spirals into the cell shape. Although these remain largely fantasies at present, the field of “cell shape engineering” in bacteria appears promising.

## 7. REFERENCES

1. Scheffers, D. J. & M. G. Pinho: Bacterial cell wall synthesis: new insights from localization studies. *Microbiol Mol Biol Rev*, 69, 585-607 (2005)
2. van Heijenoort, J.: Assembly of the monomer unit of bacterial peptidoglycan. *Cell Mol Life Sci*, 54, 300-4 (1998)
3. Holtje, J. V.: Growth of the stress-bearing and shape-maintaining murein sacculus of *Escherichia coli*. *Microbiol Mol Biol Rev*, 62, 181-203 (1998)
4. Gitai, Z.: The new bacterial cell biology: moving parts and subcellular architecture. *Cell*, 120, 577-86 (2005)
5. Shih, Y. L. & L. Rothfield: The bacterial cytoskeleton. *Microbiol Mol Biol Rev*, 70, 729-54 (2006)
6. Graumann, P. L.: Cytoskeletal elements in bacteria. *Annu Rev Microbiol*, 61, 589-618 (2007)
7. Gitai, Z., N. A. Dye, A. Reisenauer, M. Wachi & L. Shapiro: MreB actin-mediated segregation of a specific region of a bacterial chromosome. *Cell*, 120, 329-41 (2005)
8. Karczmarek, A., R. Martinez-Arteaga, S. Alexeeva, F. G. Hansen, M. Vicente, N. Nanninga & T. den Blaauwen: DNA and origin region segregation are not affected by the transition from rod to sphere after inhibition of *Escherichia coli* MreB by A22. *Mol Microbiol*, 65, 51-63 (2007)
9. Cabeen, M. T., G. Charbon, W. Vollmer, P. Born, N. Ausmees, D. B. Weibel & C. Jacobs-Wagner: Bacterial cell curvature through mechanical control of cell growth. *EMBO J*, 28, 1208-19 (2009)



## The mechanics of shape in prokaryotes

10. Lan, G., C. W. Wolgemuth & S. X. Sun: Z-ring force and cell shape during division in rod-like bacteria. *Proc Natl Acad Sci U S A*, 104, 16110-5 (2007)
11. Cabeen, M. T. & C. Jacobs-Wagner: Bacterial cell shape. *Nat Rev Microbiol*, 3, 601-10 (2005)
12. Young, K. D.: The selective value of bacterial shape. *Microbiol Mol Biol Rev*, 70, 660-703 (2006)
13. Angert, E. R.: Alternatives to binary fission in bacteria. *Nat Rev Microbiol*, 3, 214-24 (2005)
14. Dusenbery, D. B.: Fitness landscapes for effects of shape on chemotaxis and other behaviors of bacteria. *J Bacteriol*, 180, 5978-83 (1998)
15. Cooper, S. & M. W. Denny: A conjecture on the relationship of bacterial shape to motility in rod-shaped bacteria. *FEMS Microbiology Letters*, 148, 227-231 (1997)
16. Cho, H., H. Jonsson, K. Campbell, P. Melke, J. W. Williams, B. Jedynak, A. M. Stevens, A. Groisman & A. Levchenko: Self-organization in high-density bacterial colonies: efficient crowd control. *PLoS Biol*, 5, e302 (2007)
17. Borgnia, M. J., S. Subramaniam & J. L. Milne: Three-dimensional imaging of the highly bent architecture of *Bdellovibrio bacteriovorus* by using cryo-electron tomography. *J Bacteriol*, 190, 2588-96 (2008)
18. Yao, X., M. Jericho, D. Pink & T. Beveridge: Thickness and elasticity of gram-negative murein sacculi measured by atomic force microscopy. *J Bacteriol*, 181, 6865-75 (1999)
19. Francius, G., O. Domenech, M. P. Mingeot-Leclercq & Y. F. Dufrene: Direct observation of *Staphylococcus aureus* cell wall digestion by lysostaphin. *J Bacteriol*, 190, 7904-9 (2008)
20. Mendelson, N. H., J. E. Sarlls, C. W. Wolgemuth & R. E. Goldstein: Chiral self-propulsion of growing bacterial macrofibers on a solid surface. *Phys Rev Lett*, 84, 1627-30 (2000)
21. Vollmer, W. & J. V. Holtje: The architecture of the murein (peptidoglycan) in gram-negative bacteria: vertical scaffold or horizontal layer(s)? *J Bacteriol*, 186, 5978-87 (2004)
22. Verwer, R. W., N. Nanninga, W. Keck & U. Schwarz: Arrangement of glycan chains in the sacculus of *Escherichia coli*. *J Bacteriol*, 136, 723-9 (1978)
23. Verwer, R. W., E. H. Beachey, W. Keck, A. M. Stoub & J. E. Poldermans: Oriented fragmentation of *Escherichia coli* sacculi by sonication. *J Bacteriol*, 141, 327-32 (1980)
24. Gan, L., S. Chen & G. J. Jensen: Molecular organization of Gram-negative peptidoglycan. *Proc Natl Acad Sci U S A*, 105, 18953-7 (2008)
25. Dmitriev, B., F. Toukach & S. Ehlers: Towards a comprehensive view of the bacterial cell wall. *Trends Microbiol*, 13, 569-74 (2005)
26. Matias, V. R., A. Al-Amoudi, J. Dubochet & T. J. Beveridge: Cryo-transmission electron microscopy of frozen-hydrated sections of *Escherichia coli* and *Pseudomonas aeruginosa*. *J Bacteriol*, 185, 6112-8 (2003)
27. Dubochet, J., A. W. McDowell, B. Menge, E. N. Schmid & K. G. Lickfeld: Electron microscopy of frozen-hydrated bacteria. *J Bacteriol*, 155, 381-90 (1983)
28. Matias, V. R. & T. J. Beveridge: Cryo-electron microscopy reveals native polymeric cell wall structure in *Bacillus subtilis* 168 and the existence of a periplasmic space. *Mol Microbiol*, 56, 240-51 (2005)
29. Hayhurst, E. J., L. Kailas, J. K. Hobbs & S. J. Foster: Cell wall peptidoglycan architecture in *Bacillus subtilis*. *Proc Natl Acad Sci U S A*, 105, 14603-8 (2008)
30. Labischinski, H., G. Barnickel, H. Bradaczek & P. Giesbrecht: On the secondary and tertiary structure of murein. Low and medium-angle X-ray evidence against chitin-based conformations of bacterial peptidoglycan. *Eur J Biochem*, 95, 147-55 (1979)
31. Koch, A. L.: Orientation of the peptidoglycan chains in the sacculus of *Escherichia coli*. *Res Microbiol*, 149, 689-701 (1998)
32. Huang, K. C., R. Mukhopadhyay, B. Wen, Z. Gitai & N. S. Wingreen: Cell shape and cell-wall organization in Gram-negative bacteria. *Proc Natl Acad Sci U S A*, 105, 19282-7 (2008)
33. Cros, S., C. Garnier, M. A. Axelos, A. Imberty & S. Perez: Solution conformations of pectin polysaccharides: determination of chain characteristics by small angle neutron scattering, viscometry, and molecular modeling. *Biopolymers*, 39, 339-52 (1996)
34. Yousif, S. Y., J. K. Broome-Smith & B. G. Spratt: Lysis of *Escherichia coli* by beta-lactam antibiotics: deletion analysis of the role of penicillin-binding proteins 1A and 1B. *J Gen Microbiol*, 131, 2839-45 (1985)
35. Schiffer, G. & J. V. Holtje: Cloning and characterization of PBP 1C, a third member of the multimodular class A penicillin-binding proteins of *Escherichia coli*. *J Biol Chem*, 274, 32031-9 (1999)
36. Bendezu, F. O. & P. A. de Boer: Conditional lethality, division defects, membrane involution, and endocytosis in *mre* and *mrd* shape mutants of *Escherichia coli*. *J Bacteriol*, 190, 1792-811 (2008)

## The mechanics of shape in prokaryotes

37. Spratt, B. G.: Distinct penicillin binding proteins involved in the division, elongation, and shape of *Escherichia coli* K12. *Proc Natl Acad Sci U S A*, 72, 2999-3003 (1975)
38. Woldringh, C. L., P. Huls, E. Pas, G. J. Brakenhoff & N. Nanninga: Topography of Peptidoglycan Synthesis during Elongation and Polar-Cap Formation in a Cell-Division Mutant of *Escherichia-Coli* Mc4100. *Journal of General Microbiology*, 133, 575-586 (1987)
39. De Pedro, M. A., H. Schwarz & A. L. Koch: Patchiness of murein insertion into the sidewall of *Escherichia coli*. *Microbiology*, 149, 1753-61 (2003)
40. de Pedro, M. A., J. C. Quintela, J. V. Holtje & H. Schwarz: Murein segregation in *Escherichia coli*. *J Bacteriol*, 179, 2823-34 (1997)
41. Daniel, R. A. & J. Errington: Control of cell morphogenesis in bacteria: two distinct ways to make a rod-shaped cell. *Cell*, 113, 767-76 (2003)
42. Mendelson, N. H.: Helical growth of *Bacillus subtilis*: a new model of cell growth. *Proc Natl Acad Sci U S A*, 73, 1740-4 (1976)
43. Saxe, C. L., 3rd & N. H. Mendelson: Identification of mutations associated with macrofiber formation in *Bacillus subtilis*. *Genetics*, 107, 551-61 (1984)
44. Mendelson, N. H.: Regulation of *Bacillus subtilis* macrofiber twist development by D-cycloserine. *J Bacteriol*, 170, 2336-43 (1988)
45. Surana, U., A. J. Wolfe & N. H. Mendelson: Regulation of *Bacillus subtilis* macrofiber twist development by D-alanine. *J Bacteriol*, 170, 2328-35 (1988)
46. Romeis, T. & J. V. Holtje: Specific interaction of penicillin-binding proteins 3 and 7/8 with soluble lytic transglycosylase in *Escherichia coli*. *J Biol Chem*, 269, 21603-7 (1994)
47. Vollmer, W., M. von Rechenberg & J. V. Holtje: Demonstration of molecular interactions between the murein polymerase PBP1B, the lytic transglycosylase MltA, and the scaffolding protein MipA of *Escherichia coli*. *J Biol Chem*, 274, 6726-34 (1999)
48. Goodell, E. W.: Recycling of murein by *Escherichia coli*. *J Bacteriol*, 163, 305-10 (1985)
49. Park, J. T.: Identification of a dedicated recycling pathway for anhydro-N-acetylmuramic acid and N-acetylglucosamine derived from *Escherichia coli* cell wall murein. *J Bacteriol*, 183, 3842-7 (2001)
50. Shah, I. M., M. H. Laaberki, D. L. Popham & J. Dworkin: A eukaryotic-like Ser/Thr kinase signals bacteria to exit dormancy in response to peptidoglycan fragments. *Cell*, 135, 486-96 (2008)
51. Sycuro, L. K., Z. Pincus, K. D. Gutierrez, J. Biboy, C. A. Stern, W. Vollmer & N. R. Salama: Peptidoglycan crosslinking relaxation promotes *Helicobacter pylori*'s helical shape and stomach colonization. *Cell*, 141, 822-33 (2010)
52. van den Ent, F., L. A. Amos & J. Lowe: Prokaryotic origin of the actin cytoskeleton. *Nature*, 413, 39-44 (2001)
53. Bendezu, F. O., C. A. Hale, T. G. Bernhardt & P. A. de Boer: RodZ (YfgA) is required for proper assembly of the MreB actin cytoskeleton and cell shape in *E. coli*. *EMBO J*, 28, 193-204 (2009)
54. Esue, O., D. Wirtz & Y. Tseng: GTPase activity, structure, and mechanical properties of filaments assembled from bacterial cytoskeleton protein MreB. *J Bacteriol*, 188, 968-76 (2006)
55. Vats, P. & L. Rothfield: Duplication and segregation of the actin (MreB) cytoskeleton during the prokaryotic cell cycle. *Proc Natl Acad Sci U S A*, 104, 17795-800 (2007)
56. Jones, L. J., R. Carballido-Lopez & J. Errington: Control of cell shape in bacteria: helical, actin-like filaments in *Bacillus subtilis*. *Cell*, 104, 913-22 (2001)
57. Bean, G. J. & K. J. Amann: Polymerization properties of the *Thermotoga maritima* actin MreB: roles of temperature, nucleotides, and ions. *Biochemistry*, 47, 826-35 (2008)
58. Vats, P., Y. L. Shih & L. Rothfield: Assembly of the MreB-associated cytoskeletal ring of *Escherichia coli*. *Mol Microbiol*, 72, 170-82 (2009)
59. Shiomi, D., M. Sakai & H. Niki: Determination of bacterial rod shape by a novel cytoskeletal membrane protein. *EMBO J*, 27, 3081-91 (2008)
60. van den Ent, F., C. M. Johnson, L. Persons, P. de Boer & J. Lowe: Bacterial actin MreB assembles in complex with cell shape protein RodZ. *EMBO J*, 29, 1081-90 (2010)
61. Kruse, T., J. Bork-Jensen & K. Gerdes: The morphogenetic MreBCD proteins of *Escherichia coli* form an essential membrane-bound complex. *Mol Microbiol*, 55, 78-89 (2005)
62. Carballido-Lopez, R., A. Formstone, Y. Li, S. D. Ehrlich, P. Noirot & J. Errington: Actin homolog MreBH governs cell morphogenesis by localization of the cell wall hydrolase LytE. *Dev Cell*, 11, 399-409 (2006)
63. van Teeffelen, S., S. Wang, L. Furchtgott, K. C. Huang, N. S. Wingreen, J. W. Shaevitz & Z. Gitai: The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. *Proc Natl Acad Sci U S A*, 108, 15822-7 (2011)

## The mechanics of shape in prokaryotes

64. Garner, E. C., R. Bernard, W. Wang, X. Zhuang, D. Z. Rudner & T. Mitchison: Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. *Science*, 333, 222-5 (2011)
65. Dominguez-Escobar, J., A. Chastanet, A. H. Crevenna, V. Fromion, R. Wedlich-Soldner & R. Carballido-Lopez: Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. *Science*, 333, 225-8 (2011)
66. Furchtgott, L., N. S. Wingreen & K. C. Huang: Mechanisms for maintaining cell shape in rod-shaped Gram-negative bacteria. *Mol Microbiol*, 81, 340-353 (2011)
67. Wolgemuth, C. W., Y. F. Inclan, J. Quan, S. Mukherjee, G. Oster & M. A. Koehl: How to make a spiral bacterium. *Phys Biol*, 2, 189-99 (2005)
68. Wang, S., H. Arellano-Santoyo, P. A. Combs & J. W. Shaevitz: Actin-like cytoskeleton filaments contribute to cell mechanics in bacteria. *Proc Natl Acad Sci U S A*, 107, 9182-5 (2010)
69. Takeuchi, S., W. R. DiLuzio, D. B. Weibel & G. M. Whitesides: Controlling the shape of filamentous cells of *Escherichia coli*. *Nano Lett*, 5, 1819-23 (2005)
70. Mannik, J., R. Driessen, P. Galajda, J. E. Keymer & C. Dekker: Bacterial growth and motility in sub-micron constrictions. *Proc Natl Acad Sci U S A*, 106, 14861-6 (2009)
71. Adams, D. W. & J. Errington: Bacterial cell division: assembly, maintenance and disassembly of the Z ring. *Nat Rev Microbiol*, 7, 642-53 (2009)
72. Lu, C., M. Reedy & H. P. Erickson: Straight and curved conformations of FtsZ are regulated by GTP hydrolysis. *J Bacteriol*, 182, 164-70 (2000)
73. Osawa, M., D. E. Anderson & H. P. Erickson: Reconstitution of contractile FtsZ rings in liposomes. *Science*, 320, 792-4 (2008)
74. Li, Z., M. J. Trimble, Y. V. Brun & G. J. Jensen: The structure of FtsZ filaments in vivo suggests a force-generating role in cell division. *EMBO J*, 26, 4694-708 (2007)
75. Aarsman, M. E., A. Piette, C. Fraipont, T. M. Vinkenvleugel, M. Nguyen-Disteche & T. den Blaauwen: Maturation of the *Escherichia coli* divisome occurs in two steps. *Mol Microbiol*, 55, 1631-45 (2005)
76. Schmidt, K. L., N. D. Peterson, R. J. Kustus, M. C. Wissel, B. Graham, G. J. Phillips & D. S. Weiss: A predicted ABC transporter, FtsEX, is needed for cell division in *Escherichia coli*. *J Bacteriol*, 186, 785-93 (2004)
77. Buddelmeijer, N. & J. Beckwith: A complex of the *Escherichia coli* cell division proteins FtsL, FtsB and FtsQ forms independently of its localization to the septal region. *Mol Microbiol*, 52, 1315-27 (2004)
78. Bernhardt, T. G. & P. A. de Boer: The *Escherichia coli* amidase AmiC is a periplasmic septal ring component exported via the twin-arginine transport pathway. *Mol Microbiol*, 48, 1171-82 (2003)
79. Errington, J., R. A. Daniel & D. J. Scheffers: Cytokinesis in bacteria. *Microbiol Mol Biol Rev*, 67, 52-65, table of contents (2003)
80. Heidrich, C., M. F. Templin, A. Ursinus, M. Merdanovic, J. Berger, H. Schwarz, M. A. de Pedro & J. V. Holtje: Involvement of N-acetylmuramyl-L-alanine amidases in cell separation and antibiotic-induced autolysis of *Escherichia coli*. *Mol Microbiol*, 41, 167-78 (2001)
81. Dai, K., Y. Xu & J. Lutkenhaus: Cloning and characterization of ftsN, an essential cell division gene in *Escherichia coli* isolated as a multicopy suppressor of ftsA12(Ts). *J Bacteriol*, 175, 3790-7 (1993)
82. Kusaka, I.: Growth and division of protoplasts of *Bacillus megaterium* and inhibition of division by penicillin. *J Bacteriol*, 94, 884-8 (1967)
83. Goley, E. D., L. R. Comolli, K. E. Fero, K. H. Downing & L. Shapiro: DipM links peptidoglycan remodelling to outer membrane organization in *Caulobacter*. *Mol Microbiol*, 77, 56-73 (2010)
84. Cordell, S. C., E. J. Robinson & J. Lowe: Crystal structure of the SOS cell division inhibitor SulaA and in complex with FtsZ. *Proc Natl Acad Sci U S A*, 100, 7889-94 (2003)
85. Mukherjee, A., C. Cao & J. Lutkenhaus: Inhibition of FtsZ polymerization by SulaA, an inhibitor of septation in *Escherichia coli*. *Proc Natl Acad Sci U S A*, 95, 2885-90 (1998)

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