Bacterial Shape: Two-Dimensional Questions and Possibilities

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Abstract

Events in the past decade have made it both possible and interesting to ask how bacteria create cells of defined length, diameter, and morphology. The current consensus is that bacterial shape is determined by the coordinated activities of cytoskeleton complexes that drive cell elongation and division. Cell length is most easily explained by the timing of cell division, principally by regulating the activity of the FtsZ protein. However, the question of how cells establish and maintain a specific and uniform diameter is, by far, much more difficult to answer. Mutations associated with the elongation complex often alter cell width, though it is not clear how. Some evidence suggests that diameter is strongly influenced by events during cell division. In addition, surprising new observations show that the bacterial cell wall is more highly malleable than previously believed and that cells can alter and restore their shapes by relying only on internal mechanisms.

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INTRODUCTION

One of humanity's drives is to make things, allied with the curious penchant for wanting to make them larger or smaller. On the small side of this equation, the science of nanotechnology attracts people with audacious dreams. Nanotechnologists want to fabricate the tiniest structures possible or, even better, the tiniest possible machines. The overarching desire is to create molecular sized devices of strictly defined dimensions, with little variation among the new entities, and to be able to produce multitudes over and over with few failures. Of course, bacteria have been doing this for some billions of years. Despite intermittent progress, only in the last decade have we begun to understand in any real depth how they do so.

Bacterial cytoskeleton: structure composed of cytoplasmic proteins that assemble into dynamic filamentous structures on the inner surface of the plasma membrane

The question that dominated discussion in this field 15–20 years ago was, How does a bacterium find its middle? That question seems well on its way to being answered. The new fundamental question about bacterial shape is, How does a bacterium construct a cell having a defined length, diameter, and overall geometry? Technical and genetic advances have finally made this question amenable to experiment and have reinvigorated the application of new forms of microscopic visualization.

For a superb introduction to the results, interpretations, and ideas that form the foundation of the current view of how bacteria assemble well-defined structures, I recommend the overview provided by W. Margolin (51). Other reviews are similarly educational and readable and should be consulted for more detail about how bacteria select the site of cell division (10) and how they create cells that are rod-shaped (12, 15, 23, 107), coccoid (109), or spiraled (25, 104). Of special importance are those reviews describing bacterial cytoskeleton proteins that are critical for driving cell wall synthesis and regulating its localization (13, 32, 60, 75, 92).

Instead of emphasizing how bacteria create their overall shapes, the present review concentrates on how they determine their fundamental dimensions of length and diameter. Good evidence addresses mechanisms that affect cell length, but we have little idea about how bacteria set their diameter. Here, I wish to highlight some current questions and challenges, to rescue data and ideas in danger of being forgotten or ignored, and to broaden our view about the approaches available for answering questions about basic cell dimensions. Because the scope could quickly get out of hand, the discussion centers exclusively on how rod-shaped cells set their dimensions and draws primarily from research on three model organisms: *Escherichia coli*, *Bacillus subtilis*, and *Caulobacter crescentus*.

BASIC MECHANISMS FOR SHAPING A CELL

Cell shape is often stated to be determined by the cell wall, and especially by the

peptidoglycan layer. This assertion needs to be qualified and explained, because bacteria without such walls also have complicated morphologies (57, 82). Most impressively, a recently discovered wall-less organism in the Red Sea actively changes from a long straight filament to one with bumps, curls, curves, and tightly wound spirals (3). In fact, this organism can extend a straight filamentous projection slimmer than the cell body, retract it into a tightly coiled spring-like structure, and re-extend it as a straight filament—all in the space of 8–10 seconds! Clearly, complex cell morphology in these organisms is not determined by the architecture of a rigid wall. Another reason peptidoglycan cannot be considered the ultimate source of bacterial morphology is the myriad of shapes available to bacteria even though they share highly similar cell walls (107, 108). Peptidoglycan does play a critical role in cell shape because it is the mechanical means by which most bacteria fix their shape into a specific form. Yet, because peptidoglycan is highly malleable, we must conclude that peptidoglycan instantiates a shape determined by something else.

The most widely held view is that most bacterial morphology is determined by the coordinated operation of at least two mechanisms: one responsible for cell elongation and the other for division. Elongation is driven by an actin homolog (e.g., MreB or its relatives), whereas cell division is driven by the tubulin homolog, FtsZ (23, 33, 75, 92). FtsZ assembles as a ring at the middle of the cell to localize and initiate cell division (11, 51, 99); MreB is required for cells to grow as rods instead of spheres and coordinates its activities with the FtsZ ring (75, 92). Each of these cytoplasmic scaffolds is joined by additional proteins and enzymes that self-organize to direct cell wall synthesis (**Figure 1**). The cytoskeleton proteins interact with membrane-embedded proteins, which in turn interact with proteins on the external face of the membrane, among which are the penicillin binding proteins (PBPs) that synthesize and modify peptidoglycan (**Figure 1**). For simplicity's sake I denote these two mechanisms the MreB and FtsZ systems, respectively, which is the situation in gram-negative organisms such as *E. coli*. Gram-positive bacteria such as *B. subtilis* harbor additional MreB-like homologs that also participate in cell elongation and shape determination.

It is important to distinguish between mechanisms that create cell shape and asymmetry from those that take advantage of or rely on cell morphology. The creation mechanisms can be further divided into those that generate a certain shape de novo and those that maintain or replicate shape. At the moment it seems that cytoskeleton elements create different cell shapes while peptidoglycan fixes those shapes into specific and long-lasting forms. However, we must be aware that other mechanisms may organize the cytoskeleton proteins themselves and that they may be influenced by feedback from a preexisting cell shape.

MreB: the bacterial cytoskeleton protein that forms long fibrils, or cables, that wrap around the cylindrical portion of rod-shaped cells and guide the insertion of new cell wall material

FtsZ: the bacterial cytoskeleton protein that initiates and directs cell division

PBP: penicillin binding protein

CELL LENGTH

Even though bacteria display a variety of shapes, each species seems to have relatively stable dimensions. Here we are concerned only with the two basic dimensions of length and width. Of these, length is by far the easier to explain, though there remain several unknowns and curiosities.

Note first that cell elongation and cell length are two separate characteristics. Elongation is the process by which a cell becomes rod shaped, whereas length describes the end result of that process, that is, how long the cell eventually becomes. Some mechanism must create a rod-shaped cell in the first place, but this machinery may be different from that which regulates and imposes limits on the rod-shaping machinery.

Length Variation

Bacteria in a population are not exactly the same length, with a difference of 1–4% at the time of cell division for *E. coli*, a variability that depends on strain and growth rate (34, 45, 46, 52, 83). Also, length cannot be determined by simple recourse to physical forces or osmotic

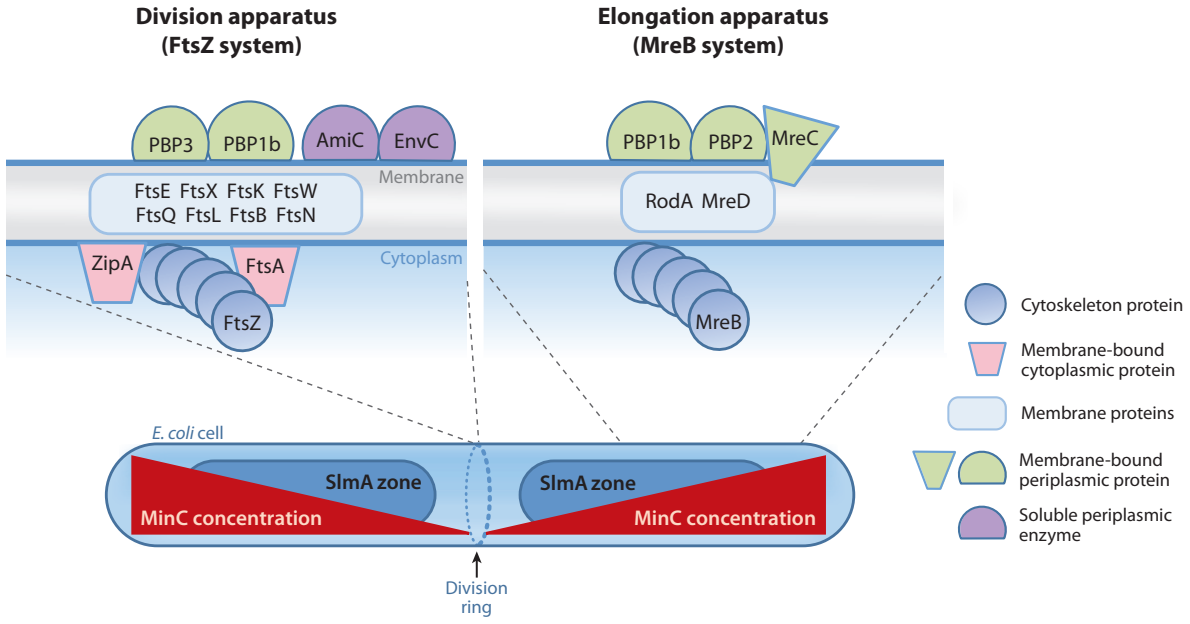

Figure 1

Composition of the division and elongation machinery in *Escherichia coli*. The division apparatus (*on the left*) and the cell elongation apparatus (*on the right*) are anchored by polymers of the FtsZ and MreB cytoskeleton proteins, respectively. Each is joined by additional cytoplasmic proteins, membrane-associated proteins, and membrane-bound periplasmic proteins or soluble periplasmic enzymes. Not all involved proteins are pictured. The identities and functions of these and other accessory proteins are described elsewhere (10, 11, 13, 32, 60, 75, 92). The bottom figure represents an elongated *E. coli* cell just prior to septation. The elongation apparatus is active along the side wall in the bulk of the cell cylinder, whereas the division apparatus is active only at the cell center. Two inhibitors of FtsZ ensure that the division ring assembles only at the cell center. When the cell grows to a sufficient length, the concentration of MinC (*in a gradient represented by the red triangle*) becomes low enough to allow assembly of the FtsZ ring. A second FtsZ inhibitor, SlmA, binds to the bacterial chromosome and inhibits FtsZ assembly in an area around the nucleoid (*central dark blue zone*).

pressure because cell length differs among closely related strains of the same species growing in the same conditions (101). Instead, there must be a mechanism that actively regulates the magnitude of this dimension. Second, as a rule, faster-growing bacteria are larger, particularly in length (69, 84). This is because the time required for chromosome replication plus the time required for cell division is constant, and faster-growing, rod-shaped cells elongate more during that time than do more slowly growing cells (110). The rate of cell division remains unchanged for some time after the rate of mass synthesis changes, so the cells at first grow much longer (or shorter) before settling into their new length (69, 102). The same phenomenon occurs in *B. subtilis* (74) and in *Acinetobacter* (40).

Growth Rate and Cell Length

An early idea to explain this length-to-growth rate connection was to posit that DNA replication initiated only when cells reached a specific mass (26, 100). Assuming that cell diameter remains constant, then shorter cells (below the critical initiation mass) will delay DNA replication until they grow longer, and longer cells (above the initiation mass) will begin DNA replication sooner. This feedback relationship should maintain daughter cell lengths within a narrow range around the mean initiation mass. A similar idea was that bacterial dimensions were proportional to the absolute concentration of DNA within a cell (42, 76). Although good to a first approximation, these schemes do not fit all circumstances (35, 97, 100). In

addition, left unexplained by these descriptive accounts was the actual mechanism by which mass or DNA concentration physically altered cell length.

Now, finally, after 50 years of study, at least one elusive molecular link between growth rate and cell length has been uncovered. Price et al. (62) noticed that *B. subtilis* mutants lacking the *ugtP* gene (then called *ypfP*) were shorter and wider. Ten years later Weart et al. (98) proved that the UgtP protein inhibits the assembly of FtsZ rings and thereby links the nutritional status of the cell to the rate of division. At high growth rates UgtP delays assembly of the septation apparatus and the cells elongate more before dividing, which explains why cells increase in length as growth rate increases. Cells from which *ugtP* is deleted are ~20% shorter, consistent with FtsZ being more active (98). How does UgtP detect and respond to growth rate? In rich medium, the concentration of uridine-5′-diphosphoglucose triggers UgtP to localize to the septum, where it comes into contact with and inhibits FtsZ (98). Cells lacking PgcA, which acts upstream of UgtP, are 35% instead of 20% shorter, hinting that one or more additional FtsZ-modulating factors may exist (98). Thus, Z ring formation and cell length are regulated by a protein whose activity varies with the amount of a metabolic intermediate, providing a satisfying explanation for how growth rate modifies cell size.

The same type of growth-to-length relationship may operate in *E. coli* because cells unable to synthesize UDP-glucose are 30% shorter than wild type (48, 97), suggesting that this metabolic intermediate also regulates growth-rate-related changes in cell size. *E. coli* has no UgtP homolog, because this organism does not produce teichoic acids, so there is probably a different FtsZ inhibitor waiting to be identified in gram-negative bacteria.

Determining Length by Regulating Division

The preceding findings suggest that cell length depends on how long the elongation apparatus is allowed to proceed before division occurs. Thus, length may be controlled primarily, if not solely, by mechanisms that enhance or delay cell division. This idea is consistent with a number of observations, most of which involve control of FtsZ assembly or function.

Nucleoid occlusion. Nucleoid positioning affects cell division by inhibiting FtsZ assembly. The *B. subtilis* Noc and *E. coli* SlmA proteins are FtsZ inhibitors that restrict septation to areas between daughter nucleoids (6, 105) (**Figure 1**). Therefore, increasing the volume of DNA or prolonging chromosomal segregation delays the appearance of a nucleoid-free zone in which FtsZ assembly can occur. Such cells become longer and cell mass increases in rough proportion to the amount of DNA. As proof that nucleoid occlusion can regulate cell length, inserting eight new Noc binding sites near the terminus of the *B. subtilis* chromosome delays division and increases length by ~16% (106).

MinCDE and analogous systems. The *E. coli* Min system restricts division to the cell center (49, 67). The activity of the MinD and MinE proteins creates a gradient of an FtsZ inhibitor, MinC, that is highest at the poles and gradually decreases toward the center of the cell (49, 64, 67) (**Figure 1**). When the cell is long enough, FtsZ assembles into a ring because the concentration of MinC at the center drops below a critical level (49, 67). Thus, cells can regulate their lengths by altering the amounts or ratios of MinC to MinDE to create the proper MinC-free zone in which division can occur. The same principle is at work in *B. subtilis* (53) and *C. crescentus* (80). It is probably a general biological mechanism because cell length in the fission yeast *Schizosaccharomyces pombe* is also determined by opposing proteins that activate or inhibit mitosis (54, 58).

Other division components. Regulation of division can also occur at the level of individual divisome components. For example, in *E. coli*, FtsA-stimulated FtsZ activity results in

Septum: the membrane and cell wall layers created during cell division that separate the two daughter cells from one another

Divisome: the complex of all proteins that assemble onto or in response to FtsZ and that are required for cell invagination leading to cell division

shorter cells (31). Specifically, an *E. coli* FtsA* mutant is 27–35% shorter because the cells divide earlier than normal, probably because FtsZ rings assemble faster (31). Also, simultaneous overproduction of FtsA and FtsZ reduces cell length by about 30% (4), perhaps by speeding the assembly of septal FtsZ rings. Similar relations between the rate of cell division and length occur in *B. subtilis*. For example, a *B. subtilis* mutant lacking the EzrA and GpsB proteins grows twice as long, and a mutant lacking PBP 1 is 50% longer (19, 59). The absence of these proteins delays septation (19), probably explaining the longer cell lengths. Longer cells are also produced in the cyanobacterium *Anabaena* when cell division is delayed by inhibiting either FtsZ or the PBP3 homolog in this organism (68). Mutations in peptidoglycan hydrolases that cut daughter cells apart at the end of division also affect cell length (85). Finally, gram-positive bacteria incorporate teichoic acids into their walls, and a mutant unable to synthesize lipoteichoic acid is longer, probably due to decreased assembly of FtsZ rings (71).

Cell elongation. The operation of the cell elongation complex, centered on MreB and its homologs, also affects cell length. In *B. subtilis*, mutations altering MreB change length (41) and *mreBH* mutants are ~50% longer (16), as are mutants lacking one or more PBPs (56). It is unlikely that these mutations enhance cell elongation, but they may slow the rate of division in ways not yet understood.

Summary. The simplest mechanism for regulating cell length is to manipulate the rate, extent, or timing of division. Many bacteria, and even yeast, alter their lengths in this way, suggesting that it is probably a general biological strategy. Because FtsZ is the principal initiator of cell division in bacteria, there are numerous examples of changing cell length by regulating when and where this protein can function. Of course, cell division requires many other proteins, and in all likelihood bacterial length is determined by the collaboration of several balancing mechanisms.

CELL DIAMETER

Making a rod-shaped cell means, first and foremost, setting a diameter. When this is accomplished, cell growth is constrained to a single dimension (length) and cellular constituents can be apportioned equally and predictably between daughter cells at cell division (108). Without a specified width, this becomes more difficult, even for spherical cells. Currently, the question of how a cell sets its diameter is the most difficult and frustrating problem in the field of bacterial shape. We know of conditions and mutations that affect cell width, but as yet nothing has coalesced into a coherent explanatory mechanism.

Although we normally think of bacteria as having a single defined width, diameter varies within a population just like any other trait. In *E. coli* and *B. subtilis*, individual cell diameters fall within 5–7% of the mean (50, 81, 101), though the diameters of the thinnest cells can be smaller by as much as 12–13% (50). Cells in different growth states also often have different shapes or sizes. *E. coli* cells in logarithmic growth are, in general, longer and wider than the same cells in stationary phase (50), and they become longer and wider when shifted from a poor to a rich growth medium (102). Other experiments confirm these generalizations (69, 86, 102, 110). What allows this variation in cell diameter, and, even more interesting, what constrains it?

Bacterial Deformation

Historical observations suggest that bacteria can squeeze through holes smaller than their own diameters (95 and references therein). These reports have met with varying levels of incredulity, and it has been relatively easy to minimize these observations by invoking poorly constructed filters, filter damage, or other technical glitches. However, a recent experiment puts new life into the idea and highlights in

dramatic fashion the extraordinary malleability of bacteria and their walls. Männik et al. (50) record the remarkable ability of *E. coli* to change its diameter and inch through a channel whose narrowest dimension is half that of a normally shaped cell. Bacteria are injected into one of two chambers connected to one another by channels of various widths and then monitored to see if cells can traverse the channels to reach a second reservoir. Surprisingly, *E. coli* cells about 0.8 μm in diameter move through slits as narrow as 0.4 μm (50).

How do cells accomplish this unanticipated feat? Initial entry and transit through the tiny channels are driven by the simple physical force of crowding. Movement into and through the channels depends not on motility but on continued growth and division, which pushes daughter cells forward. The trapped cells divide and double in mass at normal rates, indicating that growth is not impeded, but the physical constraints force cells to adopt shapes that are highly variable: bent, curved, round, branched, triangular, and other unusual morphologies. Even when mashed into these odd geometries, the septal plane during division is almost always near the geometric center of each cell, implying that FtsZ localization remains surprisingly normal. The cells are not mutants and revert to their normal shape once they reach the freedom of the second chamber.

These results provide incontrovertible proof that bacteria (or at least *E. coli*) can change shape in dramatic fashion in the normal course of adapting to a local environment, indicating that the plasticity and deformability of bacteria are greater than formerly imagined. The observations pose several difficult questions: How do cells reduce their diameter so they can enter the narrow slits? Do they enter pole first or by growing a thinner protrusion from their side wall? If they enter sideways, is the area to be extruded near the site of active septation or does it represent normal sidewall synthesis? Do different shapes arise because of problems in division or because of asymmetries in cell elongation? What happens to the cytoskeleton proteins while the cells are trapped? And how are the diameter-determining mechanisms reconstituted once the cells exit? Our inability to predict the original results, much less answer the questions they raise, emphasizes our lack of fundamental knowledge about how cells determine their dimensions.

Prosthecae: thin and often long extensions from the main body of a bacterium (also called stalks)

Diameter Control

Prosthecae. Prosthecae (also referred to as stalks) are extensions of the body of a bacterium and consist of peptidoglycan and other cell envelope components surrounding a fluid core connected to the cytoplasm. The synthesis of these structures represents an extreme example of diameter control in that such extensions are smaller in diameter than the cell body. The stalk of *C. crescentus* is approximately one-fifth the diameter of the cell and is so thin (~100- to 150-nm external diameter and ~10- to 20-nm internal diameter) (61) that it may represent the minimum width attainable by a peptidoglycan-enclosed cylindrical structure. An even more impressive feat is accomplished by *Hyphomonas neptunium*, which elaborates a thin stalk from one pole through which cytoplasm and chromosomal DNA pass to the distal end, where the stalk expands again to form a new cell body (111). Thus, the diameter of the wall alternates during the growth of a single cell, indicating there must be a mechanism for making each transition and for maintaining a specified diameter at each stage.

Prosthecae grow from the base, where they contact the main cell body (1, 72, 73), indicating that whatever determines stalk diameter is localized at this site. MreB and FtsZ are both needed to synthesize stalks (24, 63), as are PBP2 and RodA, which also participate in the growth of normal rod-shaped cells (93, 94). Mutations in these proteins increase the diameter of the stalk and decrease its rate of elongation. The conclusion is that the normal peptidoglycan-synthesizing apparatus can be co-opted to create cylindrical shapes of different diameters even within the same cell, suggesting that width is not determined by an intrinsic property of any one protein (94).

Apical growth: growth of bacterial cell wall from the tip or end of the cell, usually from a concentrated circular area

What can stalk synthesis tell us about the mechanism of diameter control? Stalk growth is a type of apical growth. That is, new peptidoglycan is inserted at one location so that stalk elongation progresses unidirectionally outward. In certain bacteria (e.g., *Streptomyces*) apical growth occurs at the poles to extend and elongate the cell. In both cases the cell wall machinery creates a ring of new peptidoglycan with a specific width, and the simplest way for this to happen would be for the machinery to be arranged as a ring of defined diameter that directs peptidoglycan synthesis evenly along its edges. The ring diameter cannot be due to any one protein, but inactivation of any constituent does alter cell or stalk diameter. For example, inactivating PBP2 increases stalk diameter at the juncture with the cell body, precisely where peptidoglycan synthesis occurs (73). Thus, we are in the confusing position of concluding that diameter cannot be determined by the intrinsic properties of any individual protein (because the same proteins synthesize cylinders of different widths) but that the correct diameter depends on each protein (because mutation of any one protein alters diameter). We are left, then, attempting to find the relevant mechanism that arranges these proteins into a machine of proper dimensions. What this might be is not at all clear.

Apical growth. Another way to think about the generation of a specific cell diameter is to consider how branches arise from bacterial cells. The filamentous *Streptomyces* species grow by polar extension (apical growth), as do the corynebacteria and mycobacteria (29). In *Streptomyces coelicolor*, a homolog of the DivIVA protein directs polar growth and accumulates in spots along the lateral wall where new branches emerge (28, 36). These branches often have smaller diameters than the original filament from which they spring (36), indicating that width is not determined by mimicking the diameter of prior peptidoglycan structures. Thus, whatever determines diameter in this organism is at work during the initial steps of branch formation, suggesting that the apparatus for wall elongation has a defined width and retains this structure as the new hyphae grow. The fact that DivIVA directs synthesis of peptidoglycan cylinders of different widths also suggests that this protein by itself is not responsible for setting the absolute diameter of the cell.

MreB and the Cell Elongation Complex

MreB. Homologs of the actin-like cytoskeleton protein MreB are found in almost all bacteria that have a nonspherical shape (14), and these cooperate with membrane proteins and peptidoglycan synthases in a complex that directs cell wall growth (13, 32, 60, 92). Mutations in many members of this complex alter cell diameter, but the plethora of effects is confusing and the mechanisms by which these proteins affect width remain mysterious.

One mechanism by which the MreB elongation complex might maintain cell diameter is by distributing peptidoglycan synthesis uniformly around the cell's circumference and along its length. However, this alone does not seem to be sufficient. For example, the rod shape of *E. coli* is not determined simply by uniform insertion of new peptidoglycan into the wall. Only when MreB directs peptidoglycan synthesis do the cells maintain their normal rod morphology (89), highlighting the primacy of this system in maintaining cell diameter.

In *B. subtilis*, MreB, Mbl, and MreBH direct the helical insertion of new peptidoglycan into the cell wall, and the cells grow as uniformly straight rods (43). This organism tolerates the loss of any one cytoskeleton protein, suggesting that the three proteins are redundant, though not completely equivalent (30, 43). When expressed at optimum levels in a mutant lacking all three cytoskeleton genes, any one protein allows the cells to grow as rods, albeit with different widths than wild-type cells (43). Therefore, overall rod shape can be directed by any of these cytoskeleton proteins, but specific widths are determined by combined action of the three.

Nevertheless, MreB remains a key component because double mutants lacking MreB and either MreBH or Mbl lose their rod shape and die (70).

MreB missense mutants. The behavior of missense variants of MreB is the best evidence that something intrinsic to the structure or activity of MreB and its polymers dictates cell width. Overexpression of cloned *mreB* alleles encoding proteins with substitutions at amino acid position 165 (normally aspartic acid) causes *E. coli* cells to grow as long filaments that vary in diameter depending on the amino acid replacement. Substituting glutamic acid or alanine decreases diameter, whereas a valine insertion increases diameter (47). MreB residue 165 is part of an ATP binding domain (47), but nothing is known about how such a change translates into diameter differences. Missense mutations in a different MreB residue have similar effects on *B. subtilis* (41). Diameter increases in cells producing an MreB variant with serine in place of leucine at position 296, or with arginine replacing methionine at residue 104 (41). As yet, nothing is known regarding how these substitutions change MreB activity or cellular diameter, but the specificity of the changes argues that investigating the structural basis for these effects should yield information about the connection between MreB and cell width.

Other proteins. Recently, three labs discovered and characterized yet another protein, RodZ, that works in conjunction with or in addition to MreB to maintain proper cell width in *E. coli* and *C. crescentus* (2, 5, 77). An *E. coli rodZ* mutant is wider (5, 77), as is a *rodZ* mutant of *C. crescentus* (2). Overproduction of MreB causes *E. coli* to become 70% wider (5), but when MreB and RodZ are overproduced simultaneously, the cells maintain a wild-type diameter (5). Thus, the ratio of MreB to RodZ may set a certain width, leading Bendezú et al. to surmise that RodZ may help create or stabilize the MreB cytoskeleton to "fine-tune the precise dimensions of cells" (5). In addition, the *B. subtilis* GpsB and PBP1 proteins promote septation and increase cell diameter (19).

Quis custodiet ipsos custodes? All these cytoskeletal alterations beg the ancient question (to paraphrase the poet Juvenal), If MreB and its homologs direct peptidoglycan insertion so that bacteria can assume their proper shape, then what directs the behavior of the cytoskeleton proteins? If MreB is no more than a track that binds periplasmic cell wall synthases and directs their traffic, what determines the shape of the underlying track itself? Cytoskeletons are not restricted to a single intracellular pattern but are dynamic and can be arranged in many ways (18, 78). There must be, then, unknown regulatory agents that respond to environmental or biochemical signals and translate these into changes in the cytoskeletal networks (17).

Summary. MreB, its homologs, and associated proteins are important in either establishing or maintaining cell diameter. At present, it is not possible to say whether they determine cell width per se or if they just specify that a cell will be rod shaped as opposed to spherical, with some other device determining actual width. In any case, we have no real clue about the molecular mechanism by which MreB or the cell elongation complex directs the formation of cells with individual diameters.

Diameter Regeneration

One way to study the mechanics of creating a specific diameter is to treat cells so that they lose the ability to control width and then observe how they re-establish their wild-type shape. In an early version of this kind of experiment, Elliott et al. (27) observed how lysozyme-generated protoplasts of *Bacillus licheniformis* regenerated into rod-shaped cells. The original amorphous spheres became highly pleomorphic and finally rod shaped (27). Shiomi et al. (77) watched a spherical *E. coli rodZ* mutant elongate into large and bloated rod-like

shapes with irregular widths, regaining normal rod shape after 4–5 h of growth. Another approach is exemplified by the squashed cells created when *E. coli* is forced into channels with tiny diameters (50). Cells released from their confining channels are extremely aberrant, implying that the process of changing their diameter seriously perturbs the normal mechanisms of shape control, but proliferation in an unconfined space gives rise to cells with wild-type dimensions (50). The conclusion is that the shape- and diameter-determining mechanisms are flexible (*E. coli* can be forced into significantly different shapes) and resilient (the mechanisms spring back to produce the original shapes).

A recent set of experiments investigates diameter regeneration and opens a host of new questions. Takacs et al. (79) inactivated MreB in *C. crescentus* so that the organism transforms from a thin, crescent shape into much wider, lemon-shaped or spherical cells. During recovery, cells return to their normal width in a surprising manner. Bloated cells elongate and gradually reduce their diameter until they reach the wild-type width, at which time the original cell has become a very long, thin filament (79). The process is not restricted to a specific region of the cell but instead proceeds along the length of the entire cell body. One implication is that width can be reduced against what is presumed to be a large internal turgor pressure, just the sort of physical consideration that one would have thought would enlarge the cell wall. Another implication is that, at least for *C. crescentus*, a set diameter can be re-established without the need for completing intervening rounds of cell division. Questions abound. Does MreB (or any supporting protein) form cytoplasmic fibers that pull the cell wall inward? Are cell elongation and thinning due to the insertion of newly synthesized peptidoglycan precursors or to remodeling of pre-existing material? How is peptidoglycan inserted into the cell wall without MreB? Finally (and most vexing!), how does the MreB-based machinery know to halt cell thinning when the original diameter is achieved?

These experiments argue strongly that the final shape of a regenerated rod-shaped cell does not depend on the presence of a pre-existing template. Instead, cells are reshaped by de novo peptidoglycan synthesis, although it takes time and growth before the new structure is established.

SETTING DIAMETER DURING CELL DIVISION?

There are two basic, though not mutually exclusive, possibilities for when and where the cell sets its diameter—either during the process of cell division or during cell elongation. Despite data favoring the elongation complex, there is a bit of innuendo that diameter control is exerted as cells divide rather than determined during actual cell elongation.

E. coli cells grown for half a division cycle in the absence of functional PBP2 synthesize new poles with increased diameters compared to older poles, indicating that PBP2 helps to set or maintain cell width during cell division (22). Overexpression of FtsA inhibits *E. coli* cell division, and the resulting filaments enlarge at prospective septation sites (96). In addition, *E. coli* cells are not perfectly uniform cylinders capped at either end by identical hemispherical poles; instead, one pole is often slightly larger than the other (39), implying that width differences arise during the creation of new poles.

Formstone & Errington observed that the diameter increase in an *mreB*-null mutant of *B. subtilis* "appeared to occur at or near new division sites, whereas the old poles retained a constant width" (30). Similar phenomena occur when *B. subtilis* cells are depleted of YlaN so that they become wider at new division sites and form tapered daughter cells (38), and when Mbl is gradually depleted from *B. subtilis* so that peptidoglycan synthesis is shifted toward developing septa (43). Also, *B. subtilis ugtP* mutants are both shorter and wider (62, 98). Because cell length in this mutant is controlled by expressing an FtsZ inhibitor, FtsZ may also be responsible, directly or indirectly, for

determining cell width. All these examples hint that diameter change occurs during the division cycle.

When RodZ is overproduced in *C. crescentus*, the cells bulge outward at their septa and have long thin connections between daughter cells, suggesting a division defect (2). Underscoring this relationship, RodZ colocalizes with FtsZ at the septum when division begins, after which it disperses (2). Thus, RodZ, in addition to its interactions with MreB, may exert some of its diameter-related effects during cell division.

The best counterexample for arguing that diameter is determined during elongation rather than division is the recent observation by Takacs et al. (79) that spherical *C. crescentus* cells recover wild-type diameter in the absence of cell division. It is, of course, possible that this is a special circumstance or, perhaps more likely, that the two systems collaborate to determine cell width.

POSSIBLE DIVISION-MEDIATED MECHANISMS

How might diameter be set during division? One way a cell could control its width would be to synthesize an internal hoop of defined diameter to direct cell wall synthesis (107). FtsZ forms a ring at the cell's midpoint and directs peptidoglycan synthesis during division, so if a minimum amount of FtsZ is required to encircle the cell before division can initiate, then diameter might be maintained within a certain range (108). Although this may explain how cells retain a maximum width, it does not explain why a cell is a particular width to begin with nor does it explain why cell diameter does not gradually decrease since, in principle, FtsZ can form rings and initiate division in smaller cells. As noted, cells forced into microchannels adopt smaller diameters but continue to grow and divide (50), proving that FtsZ by itself does not set the initial diameter.

Recently, another potential diameter-determining hoop-like structure was observed. Vats & Rothfield (90) found that when the FtsZ ring initiates cell division, MreB forms two rings on either side that, as the cell invaginates, move into the interior of the newly forming daughter cells. These para-septal rings include MreC, MreD, PBP2, and RodA as well (91). Vats et al. (90, 91) speculate that these complexes may insert new peptidoglycan between the two rings, thereby moving them in opposite directions and away from the developing division site and poles. If true, then these para-septal rings may synthesize peptidoglycan with a defined diameter, which in turn might limit cell width during elongation prior to the subsequent division. Such a mechanism could connect diameter determination (during cell division) with diameter maintenance (during elongation).

One other division-related event might play a role in setting bacterial diameter. PIPS (PBP3-independent peptidoglycan synthesis) represents a period of FtsZ-driven cell wall synthesis that occurs immediately prior to assembly of the divisome in *E. coli* (21) and in *C. crescentus* (1). PIPS-related FtsZ activity (1, 87, 88) might affect peptidoglycan synthesis in the lateral cell wall and alter diameter. There is scant evidence for how PIPS might contribute to pole creation or cell shape. However, when FtsZ is inhibited so that normal division cannot occur, *E. coli* cells are wider near their center or at one pole and decrease in diameter toward the other pole (102). Similarly, the width of an *E. coli dnaX* mutant increases by 30%, with the increase beginning near the cell center (103). Thus, changes in cell diameter appear to originate near incipient septal sites even in the absence of cell division, implying that diameter alterations may be mediated by PIPS or by the cell elongation apparatus.

PBP3-independent peptidoglycan synthesis (PIPS): peptidoglycan synthesis driven by FtsZ prior to cell wall invagination during division

Summary

A variety of observations in different organisms supports the idea that diameter changes originate during cell division or at those positions where division is slated to occur. The basic concept that pole diameter might constrain cell diameter was proposed first by Koch (44). The concept deserves reinvestigation.

OTHER PHENOTYPES, MORE QUESTIONS

New Mutants

There are, without doubt, undiscovered factors that help regulate cell diameter. For example, several newly isolated mutants change the diameter of *C. crescentus* (A. Alyahya & C. Jacobs-Wagner, personal communication). One, a transposon insertion into a gene that encodes polyhydroxyalkanoate synthase almost doubles cell diameter and reduces cell curvature. A second insertion increases cell width and affects a gene that encodes a protein associated with cobalamin synthesis. Polar effects on downstream genes have not yet been ruled out, but regardless of their final characterization the mutations affect pathways that were not predicted to be involved in regulating cellular morphology. Such surprises underscore how little we really know about how cell width is generated or modified. Hopefully, such examples will inspire more investigators to at least look at their cells to see if their own mutants affect these dimensions.

Teichoic Acids

The walls of gram-positive bacteria are composed of approximately equal amounts of peptidoglycan and a set of covalently linked anionic polymers, the teichoic and teichuronic acids (8, 55). Although these anionic polymers contribute to overall cell shape, their morphological role has been studied far less than that of peptidoglycan. In fact, one of the first identified morphogenes to cause *B. subtilis* cells to lose their rod shape encoded an enzyme in the teichoic acid synthetic pathway (37, 66). More recent work proves that *B. subtilis* cells lacking wall teichoic acid have dramatic shape defects, including increases in diameter, bends, bulges, septation abnormalities, and uneven wall thickening (7, 9, 20). In contrast, when lipoteichoic acid is absent cell diameter is reduced (71). The biosynthetic pathways for synthesizing peptidoglycan and teichoic acids share undecaprenyl-pyrophosphate as a membrane carrier (8), so it is conceivable that effects attributed to one pathway could be caused by a concomitant alteration of the other. In any case, these relationships need to be dissected more completely.

Peptidoglycan Precursor Imbalances

Depletion of the MurB protein causes *B. subtilis* cells to widen, with the increase often associated with a septation site (65). Similarly, a sublethal concentration of bacitracin eliminates cell widening when FtsA is overexpressed (96). Both conditions interfere with the synthesis of peptidoglycan precursors, begging the question of why cell diameter is sensitive to alterations in the concentration of these substrates.

CONCLUDING REMARKS

The most macroscopic characteristics of bacteria are their length and diameter, and at the moment we can reliably explain only one. However, it seems the major rules have been laid down: Most cells contain at least two major cytoskeleton structures that drive cell elongation and division; altering these complexes can affect length and width; and basic cell dimensions as well as overall shape are cemented in place by cell wall synthesis directed by cytoskeleton guides. For all its ancient antecedents, the field of bacterial morphology is a young one, full of fundamental questions and observations that need to be revisited and incorporated into this new framework. One thing seems certain: Bacterial shape depends on but is not solely determined by physical forces. Instead, bacteria actively manipulate their dimensions and morphology. Future work will hopefully bring us closer to understanding how the underlying machinery generates cylindrical tubes with particular and uniform diameters, how bacteria specify their dimensions, and how cells initiate and control transient and reversible morphological change.

SUMMARY POINTS

1. Basic bacterial morphology is determined by a collaboration between two cytoskeleton systems: the cell elongation apparatus, based on MreB and its homologs, and the cell division apparatus, based on FtsZ and other divisome proteins. Additional cytoskeleton proteins direct the formation of more elaborate morphologies.
2. Bacterial cell length is determined most likely by the frequency of cell division. The best examples of such regulation are those proteins that inhibit the assembly of FtsZ based on cellular geometry (MinC), chromosome location (SlmA and Noc), and growth rate (UgtP).
3. Proteins associated with the MreB cell elongation complex help to determine cell diameter, and mutations that eliminate or create variations in these components can either increase or decrease cell width or eliminate all width restrictions.
4. Cellular diameter is changed during either cell elongation or cell division. Although not certain, many observations hint that diameter alterations arise during cell division.
5. The mechanisms for setting any one diameter or changing between diameters are not known, and the principles underlying diameter determination are frustratingly difficult to imagine.
6. Bacterial cell walls are highly malleable, and the mechanisms for shape determination can be perturbed physically or genetically to give rise to cells with extremely variable morphologies. Nonetheless, these internal mechanisms can reconstitute themselves and return cells to their normal shapes.
7. Many proteins, cell wall components, and growth conditions affect cell length and diameter, but few can be accommodated into currently known schemes of shape control.

FUTURE ISSUES

1. How does the cell elongation apparatus contribute to length determination?
2. If a cytoskeleton scaffold controls cell width by guiding the cell wall synthases, then what determines the shape of the underlying cytoskeleton track itself?
3. Do other division inhibitors respond to metabolic or environmental cues and signals?
4. At what stage of the cell cycle is its diameter set?
5. How many other proteins are involved in setting basic bacterial cell dimensions?
6. Is there a way to select or enrich for mutants that have altered diameters so that new components involved in setting cell width can be identified?

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