

MicroReview

Bacterial shape

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'Architecture is the adaptation of form to resist force'.
John Ruskin (1874)

Summary

In free-living eubacteria an external shell of peptidoglycan opposes internal hydrostatic pressure and prevents membrane rupture and death. At the same time, this wall imposes on each cell a shape. Because shape is both stable and heritable, as is the ability of many organisms to execute defined morphological transformations, cells must actively choose from among a large repertoire of available shapes. How they do so has been debated for decades, but recently experiment has begun to catch up with theory. Two discoveries are particularly informative. First, specific protein assemblies, nucleated by FtsZ, MreB or Mbl, appear to act as internal scaffolds that influence cell shape, perhaps by correctly localizing synthetic enzymes. Second, defects in cell shape are correlated with the presence of inappropriately placed, metabolically inert patches of peptidoglycan. When combined with what we know about mutants affecting cellular morphology, these observations suggest that bacteria may fabricate specific shapes by directing the synthesis of two kinds of cell wall: a long-lived, rigid framework that defines overall topology, and a metabolically plastic peptidoglycan whose shape is directed by internal scaffolds.

Introduction

At present, we cannot explain in comfortable detail how even one bacterium constructs itself, much less how the vast constellation of prokaryotic shapes arises. Our ignorance is broad and profound, and encompasses the most basic of questions. For example, out of the universe of

possibilities, how do cells select, create and maintain a defined size, length and width? Why are the dimensions of most cells uniform instead of irregular? How do unevenly shaped cells manage to endow their descendants with reproducible irregularities? Why do some cells branch profusely and others not at all? And how do cells preserve or adjust these parameters during growth, or when they enter and exit different environments, or as they progress through a developmental cycle?

Unfortunately, especially when compared with advances in other physiological arenas, the answers to these questions have changed relatively little in over 30 years since the incisive reviews of Henning and Schwarz, who charted the broad outlines of the problems and their solutions (Henning and Schwarz, 1973; Henning, 1975). Subsequent treatments re-emphasized the problems and illuminated pieces of the puzzle (Harold, 1990; Nanninga, 1998), but despite a more thorough knowledge of peptidoglycan synthesis and bacterial cell division, our understanding of morphological control remains dim and untidy. Happily, recent discoveries are fleshing out previously vague ideas, and substantial progress is being made in identifying the molecular mechanisms responsible for building cells of diverse, but individual, shapes.

Scaffolds

In eubacteria, the load-bearing structure is peptidoglycan, an interlaced monomolecular meshwork that defines cell shape (Höltje, 1998; Nanninga, 1998). Because the chemical composition of peptidoglycan is similar among cells of diverse shapes, individual building blocks cannot explicitly define the form of the wall (Schwarz and Leutgeb, 1971; de Pedro *et al.*, 2003). But if the shape of the prokaryotic cell wall does not appear spontaneously then its form must be directed, and for over 40 years the question has been, 'By what?' As in any construction project, there are only two general ways to define the boundaries of a wall – by internal or external scaffolding.

Internal scaffolding

Consider the problem of setting the extent and uniformity of a cell's diameter. There are three ways to do this

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(Fig. 1). Faithful replication of a series of hoops having defined radii could define the boundaries of the side walls in a rod-shaped organism (Fig. 1, top) or a spiral of defined pitch could accomplish the same effect (Fig. 1, middle). However, to set the diameter *de novo* by either of these two mechanisms would require a chemically determined self-assembly of the hoop or spiral components. If such self-directed assembly does not occur, then pre-existing structures must act as templates to dictate the diameter of the replicas. The third way to inscribe a structure of defined radius is to use a compass, a device with one stationary end attached to another that swings freely to describe the circumference of a circle. As a biological example, imagine molecular 'spokes' of defined and equal length attached to a central linear polymer (Fig. 1, bottom). If the distal ends of the spokes were embedded in the cytoplasmic membrane, osmotic pressure would extend the membrane to the limits of the spokes, creating a rod-shaped organism (Fig. 1, bottom).

Interestingly, prokaryotes seem to use all three mechanisms. The FtsZ protein is the earliest known component of a ring (hoop) required for septation (Buddelmeijer and Beckwith, 2002; Lutkenhaus, 2002). However, the diame-

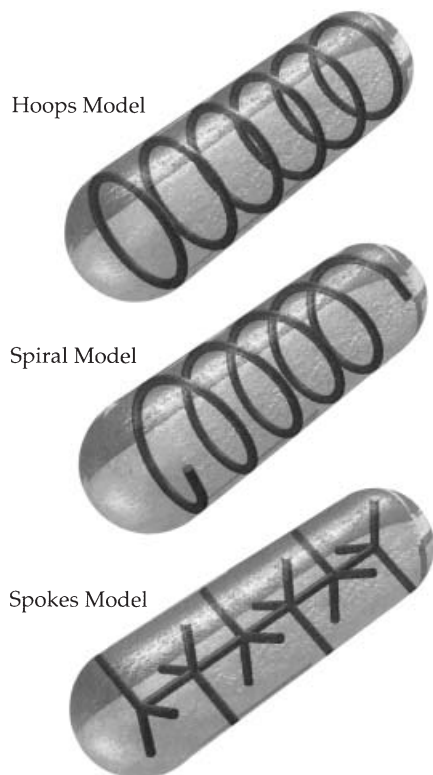


Fig. 1. Models of possible shape-defining scaffolds. Each schematic of a rod-shaped bacterium contains one of three different internal structures. Cell diameter is determined by one or more hoops of defined radius (top), by a spiral of defined pitch (centre), or by an axle-and-spoke arrangement (bottom).

ter of this structure is not set by self-assembly because the Z ring contracts during cell division and incomplete arcs can initiate septation (Addinall and Lutkenhaus, 1996; Pas *et al.*, 2001; de Pedro *et al.*, 2001). Also, FtsZ polymerizes *in vitro* as straight filaments, flat sheets or tiny rings (Lutkenhaus and Addinall, 1997; Lu *et al.* 2000). Thus, the Z ring does not define an independent radius, though it may help retain or reproduce an existing diameter.

Potential spiral scaffolds are constructed by the MreB and Mbl proteins of *Bacillus subtilis* and the MreB homologue in *Escherichia coli*, which assemble on the cytoplasmic face of the inner membrane (Wachi *et al.*, 1987; Jones *et al.*, 2001; Carballido-Lopez and Errington, 2003). Mutations in these proteins cause bacteria to grow as abnormal spheroids or misshapen rods, indicating the proteins regulate cell shape (Abhayawardhane and Stewart, 1995; Jones *et al.*, 2001). The diameters of these spirals are also not self-generated: the helical pitch of Mbl polymers changes with cell length and protein concentration (Carballido-Lopez and Errington, 2003), and MreB filaments created *in vitro* have variable curvatures that differ from the innate diameter of the bacteria from which the proteins originate (van den Ent *et al.*, 2001). Therefore, these filaments, like the Z ring, probably assemble along the inner surface of pre-existing membrane (Carballido-Lopez and Errington, 2003). FtsZ itself may behave as a transient spiral scaffold because evanescent Z spirals appear at the end of septation, and overproduction of FtsZ-GFP produces long-lived helices (Lutkenhaus and Addinall, 1997; Sun and Margolin, 2001; Lutkenhaus, 2002).

As for the third mechanism, so far the axle-and-spoke structure has been observed in only one organism, *Mycoplasma pneumoniae*, which has no peptidoglycan or other external cell wall to maintain its unique shape. This microbe has no MreB-like internal scaffold (Jones *et al.*, 2001). Instead, ultrastructural analysis reveals that the rod-shaped extension of *M. pneumoniae* appears to be defined and supported by an internal 'blade-like rod' with spokes attached to a 'wheel-like complex' (Hegermann *et al.*, 2002), conceptually like that illustrated in Fig. 1 (bottom).

Osmotic pressure as internal scaffold

One often-discussed fourth alternative is that turgor pressure created by osmotic force dictates cell shape, obviating the need for any more complex machinery. The Surface Stress Theory (Koch, 1988) proposes that the cytoplasmic membrane behaves as an internal scaffold (actually, a fully formed mould), the shape of which is determined by nothing more than osmotic pressure and two stable poles of defined diameter. Thus, cell

shape is just a matter of synthesizing peptidoglycan onto the outer surface of the membrane to form a rigid wall.

Although the physics of turgor pressure are important and must influence the way cells and enzymes operate (Koch, 1988; 2000), the Surface Stress Theory has three major problems in trying to explain cell shape. First, the theory assumes the existence of cellular structures (inert poles of defined diameter) whose mechanism of synthesis is not generated within the theory itself, leaving unaddressed a major determinant of cell shape. Second, the theory is at odds with the behaviour of mutants, past and present, whose shape changes dramatically without altering either the poles or internal pressure. In particular, recent appreciation of the roles of the MreB and Mbl proteins indicates that cells play a more active role in shape determination than envisioned by the original theory. Finally, and most telling, the theory does not account for the spectacular diversity of prokaryotic shapes and sizes. Although osmotic pressure must play a critical role, we must look elsewhere for the specific mechanisms by which a cell adapts its form in response to this force.

External scaffolding

In principle, shape-defining hoops or spirals could be placed just as easily outside the cytoplasmic membrane. In fact, peptidoglycan is often envisioned as arranged as a series of parallel hoops, whose precise replication determines and maintains a cell's diameter (Höltje, 1998). Unfortunately, there is no direct experimental proof for such hoops. Instead, according to current data, individual glycan polymers are so short that to encircle *E. coli* once would require 200–300 interconnected chains (Höltje, 1998), raising the question of how a specific diameter could be defined or maintained. Another possibility is that peptidoglycan might be roughly spiral (Mendelson, 1982), a concept which may gain new support with recent speculations about the role of Mbl helices in peptidoglycan synthesis (Carballido-Lopez and Errington, 2003). However, even if true, such helical peptidoglycan would owe its existence to an internal scaffold instead of being an independent determinant of cell shape.

Another possible external scaffold in Gram negative eubacteria is the outer membrane. In *E. coli*, certain lipoproteins link peptidoglycan to this membrane, and the strength of this association might contribute to the maintenance of cell length (Cooper, 1991). Such a relationship is difficult to prove, but mutation or inactivation of several outer membrane components does alter cell shape. For example, an *ompA/lpp* double mutant and some *lpp* mutants are spherical (Sonntag *et al.*, 1978; Hiemstra *et al.*, 1987), and overexpression of the NlpI lipoprotein

induces cells to form 'prolate ellipsoids' whose poles are more pointed than normal (Ohara *et al.*, 1999). Also, degradation of the OmpA protein by neutrophils destroys the uniform shape of *E. coli*, creating cells that appear as irregularly shaped, flattened discs (Belaouaj *et al.*, 2000). Clearly, the outer membrane or its proteins affect cell shape. However, we cannot say whether the effects arise from the disruption of an external scaffold or by interference with the activities of peptidoglycan-specific enzymes.

Among these uncertainties is one excellent example of a structure that acts as an external skeleton. *Borrelia burgdorferi*, normally shaped like a flattened spiral, loses its undulating shape and becomes a straight rod upon inactivation of the *flaB* gene, which eliminates a set of periplasmic flagella (Motaleb *et al.*, 2000). Evidently, interaction between the flagella and peptidoglycan forces an otherwise rod-shaped cell to adopt this wavy morphology (Motaleb *et al.*, 2000). Although this is the best-documented external mechanism for altering the gross morphology of a cell, the original shape and diameter of the wall is not dictated by the flagella. Therefore, the underlying shape must be derived from some other mechanism.

FtsZ and cell shape

It seems those prokaryotes we know best rely heavily on internal scaffolds to create stable, heritable shapes. However, instead of behaving as rigid skeletons, preliminary evidence suggests these structures direct the positioning of peptidoglycan-synthesizing enzymes, which in turn construct the cell's rigid external shell (Carballido-Lopez and Errington, 2003; Kroos and Maddock, 2003). We know for certain FtsZ performs this function. Bacterial division is initiated at a cell's mid-point by formation of a polymeric ring of FtsZ, onto which a cascade of proteins assembles to direct invagination of the envelope (Budde-meijer and Beckwith, 2002). As part of this process, the penicillin-binding protein (PBP) 3 (FtsI) redirects peptidoglycan synthesis from a diffuse incorporation throughout the cylindrical part of the wall (directed by PBP 2) to a localized incorporation at the developing septum (Höltje, 1998; Nanninga, 1998). A simple, though speculative, explanation for this cycle is that association with the Z ring activates PBP 3 so that it briefly out-competes PBP 2 for substrates or other components of the synthetic apparatus. In any case, FtsZ polymerization constitutes the specific, recurring signal that directs the location and synthesis of septal peptidoglycan.

Abundant indirect evidence supports the idea that FtsZ also affects overall cell shape. A long history of secondary observations indicates that aberrantly shaped *E. coli* are associated with treatments or mutations that interfere with cell division, DNA replication and peptidoglycan synthe-

sis. Although the variety of causation makes it difficult to discern a common mechanism, reinterpreting the results in light of what we now know suggests that altering the position or activity of FtsZ may be the unifying theme.

FtsZ

Strong evidence of a role for FtsZ comes from the serious morphological anomalies that arise in certain FtsZ mutants of *E. coli*. For example, at the restrictive temperature, an *ftsZ2(ts)* mutant grows as branched filaments, short Y-forms and cells with polar protrusions, and an *ftsZ26(ts)* mutant forms Y-shaped cells or cells with malformed poles (Bi and Lutkenhaus, 1992; Addinall and Lutkenhaus, 1996). A slight increase in the amount of mutant FtsZ84 protein produces branching in 2–10% of *E. coli* cells (Yu and Margolin, 2000), and overproduction of another missense FtsZ mutant also leads to blebs and branching (J. Stricker, pers. comm.). Even wild-type FtsZ produces morphological effects in some instances. Overproduction of the *Rhizobium meliloti* FtsZ2 protein in *E. coli* creates curved, coiled and spiral-shaped cells (Margolin and Long, 1994), overproduction of FtsZ in *R. meliloti* produces a population in which nearly all cells have bulges or knobs, are Y- or T-shaped, or are multiply branched (Latch and Margolin, 1997), and constitutive FtsZ expression produces bifurcated stalks in *Caulobacter crescentus* (Quardokus *et al.*, 1996). Taken together, the results imply that a common FtsZ-dependent mechanism generates shape abnormalities and branching across genera.

Septation proteins

Once FtsZ initiates the process, the rest of the septal ring assembles by successive incorporation of additional proteins (Buddelmeijer and Beckwith, 2002). Mutation of some of these also gives rise to shape defects. For example, a truncated *ftsA* gene product produces highly curved C-shaped cells, some of which form long, convoluted, corkscrew filaments (Gayda *et al.*, 1992), and cells overproducing four different carboxy terminal deletion mutants of FtsA are similarly curved and coiled (Yim *et al.*, 2000). Also, an FtsL mutant (another ring component) grows as Y-shaped and branched cells (Guzman *et al.*, 1992). Interestingly, Z rings are destabilized in the absence of the early septation proteins ZipA or FtsA (Pichoff and Lutkenhaus, 2002), leaving open the possibility that the shape effects of defective septal rings may be explained by abnormal activity of FtsZ.

Min proteins and DNA replication

Accurate localization of FtsZ rings also seems important for proper cell shape. Normally, polymerization and place-

ment of FtsZ is controlled by the MinCDE proteins, which inhibit Z-ring formation at the poles, and by the positions of chromosomes, which restrict Z-ring formation to nucleoid free areas (Yu and Margolin, 1999; Lutkenhaus, 2002). Therefore, interfering with either of these two regulatory mechanisms may alter cell shape indirectly via mislocalization of complete or partial Z rings.

Numerous observations argue that these two systems play some role in cell shape. For example, in a *minB (minCDE)* deletion mutant, 2–8% of the cells are branched, knobbed, kinked or filamented (Åkerlund *et al.*, 1993). Of special note is that the earliest reports of morphological alterations in *E. coli* are associated with treatments that inhibited DNA replication (see References in Åkerlund *et al.*, 1993). Thymine starvation of a *thy* strain produced cellular 'monsters', including short filaments with knobs, bulges, kinks and branches (Zaritsky, 1977), and mitomycin C-treated *E. coli* C cells exhibited knobs, bifurcations and internal bulges (Suit *et al.*, 1967). When Åkerlund *et al.* (1993) altered chromosomal dynamics by driving DNA replication from an *intR1* origin, swollen and irregular cells appeared with branches, knobs and kinked filaments. Similarly, inhibiting DNA synthesis in *Agrobacterium tumefaciens* caused every cell to elongate and branch (Fujiwara and Fukui, 1974), and 50% of mitomycin C-treated *R. meliloti* cells were branched (Latch and Margolin, 1997). Åkerlund *et al.* (1993) speculated that such misshapen cells might arise because 'aberrantly located nucleoids...interfere with the formation of a functional and properly oriented septation site' (Åkerlund *et al.*, 1993). Consistent with this interpretation, thymine-starved mutants display asymmetric invaginations in nucleoid-free regions (Woldringh *et al.*, 1994), and Z rings form only between nucleoids in a *min* mutant and in a *parC* mutant defective in chromosomal segregation (Yu and Margolin, 1999). An easy way to reconcile these diverse observations is to imagine that Min mutants or imprecise chromosome segregation create these morphological oddities via the same secondary effect of aberrant Z-ring localization.

Peptidoglycan synthesis

Whatever mechanism directs cell shape does so by synthesizing the rigid peptidoglycan exoskeleton. Therefore, anything altering peptidoglycan formation could conceivably affect cell shape. It is a surprise, then, that few mutations of the high molecular weight penicillin-binding proteins (HMW PBPs) yield morphologically abnormal cells. One PBP 3 mutant produces pointed poles in *E. coli* (Taschner *et al.*, 1988), and deletion of one or more HMW PBPs from *B. subtilis* produces abnormal cell shapes (Popham and Setlow, 1996; Pedersen *et al.*, 1999; McPherson and Popham, 2003). Interestingly, in the case of a *B. subtilis* mutant lacking PBP 1, FtsZ rings are

localized improperly or are structurally aberrant in over 20% of the cells (Pedersen *et al.*, 1999) so that, once again, mislocalization of Z rings is correlated with shape abnormalities.

Peptidoglycan modification: the worst shapes

At this point we need to consider a class of mutants that do not yet have a demonstrated connection to FtsZ. Although the mutants described above display a wide range of morphological oddities, none exhibit the extraordinary range of shape aberrations of *E. coli* lacking multiple low molecular weight (LMW) PBPs (Denome *et al.*, 1999; Nelson and Young, 2000; Nelson and Young, 2001). Individual mutants display a random assortment of cell shapes that mirror the characteristic forms of many different prokaryotes (Fig. 2). Some cells are short rods (cocco-bacilli), some are small spheres (classic cocci), some are multiply branched (actinomycetes and streptomycetes), some are highly irregular or produce bifurcated Y-forms (bifidobacteria), some are star shaped (stella), others are curved or flat on one side but cylindrical on the other (caulobacters) and some are completely unlike all of these (Fig. 2). In most cases, a cell's diameter varies dramatically along its length, at times mimicking the distinctive features of prosthecate bacteria. Overall, the shapes range from the remarkably odd to the possibly unique and illustrate the variety of shapes accessible to this single prokaryote. It is easy to imagine this diversity represents a substantial fraction of the bacterial 'shape universe' – those shapes available to any bacterium. If so, then perhaps the constellation of naturally occurring bac-

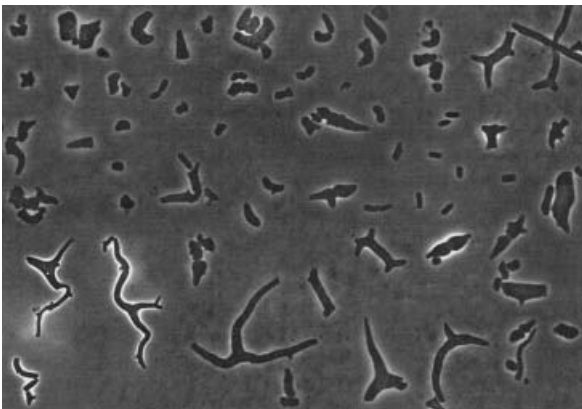


Fig. 2. Diversity of shapes in *Escherichia coli* penicillin-binding protein mutants. Photos of selected cells were compiled from several different strains lacking multiple PBPs, the important common denominator being that all lack PBP 5 (Denome *et al.*, 1999; Nelson and Young, 2000; Nelson and Young, 2001). Any one strain produces an assortment of shapes, and no one shape is peculiar to a particular mutant. Though always irregular, most of the cells in any population are more like wild-type *E. coli* than those presented here. The collage simply illustrates the variety of shapes available to these cells.

terial shapes arises by variations on a common morphological pathway.

Because the LMW PBPs are not essential for *E. coli* viability (Denome *et al.*, 1999), why does eliminating these proteins elicit changes in bacterial morphology so much greater than in any other mutants so far observed? The enzymes neither polymerize nor cross-link peptidoglycan, but instead modify it by removing the terminal D-alanine from the ends of peptide side-chains (DD-carboxypeptidases: PBP 5, PBP 6 and DacD) or by cleaving cross-linked side-chains (endopeptidases: PBPs 4 and 7) (Höltje, 1998). Therefore, these proteins must influence cell shape by simple mechanisms: the DD-carboxypeptidases by regulating the number of pentapeptide side-chains available for cross-linking during peptidoglycan synthesis, and the endopeptidases by determining the number of intact cross-links that persist in the mature sacculus. The idea that these PBPs affect morphology via an FtsZ-dependent process is developed more fully below.

The 'inert patches' idea

The foregoing considerations make it easy to visualize how misplaced or defective Z rings create oddly shaped cell poles by inaccurate septation. What is less clear is how FtsZ might create more diverse morphological defects (blebs, internal kinks, bends, curves, T-shapes, branching, diameter inconsistencies, etc.). In the following discussion, I present the framework of an idea that brings together these disparate themes and observations. The thing to keep in mind is that cells must solve two problems: they must first establish their overall shape and then maintain it. The premise presented here is this: cells establish their gross morphology by an FtsZ-dependent mechanism that synthesizes strategically placed patches or swaths of inert (stable) peptidoglycan; and they maintain their shape by a scaffold-dependent mechanism that directs the synthesis of metabolically flexible peptidoglycan.

Inert peptidoglycan (iPG) at bacterial poles

Peptidoglycan synthesis in *E. coli* and in *B. subtilis* proceeds by diffuse intercalation of new material along the length of each cell (Merad *et al.*, 1989; Woldringh *et al.*, 1987; de Pedro *et al.*, 1997). However, peptidoglycan at the poles is stable – once septation is completed, new material is not inserted and polar peptidoglycan is either not recycled or is recycled at an extremely low rate (Clarke-Sturman *et al.*, 1989; de Pedro *et al.*, 1997). No one knows what makes the poles inert. Although the chemical composition of the poles seems to be similar to the rest of the wall, their three-dimensional structure could be different in ways we are unable to detect. Or, the polar domain may be sequestered from synthetic and hydrolytic

enzymes, an idea supported by the fact that outer membrane over the poles is as stable as is peptidoglycan (de Pedro *et al.*, 1997).

de Pedro *et al.* (1997) dramatically advanced the measurement and understanding of this inert material by developing a sensitive method for tracking stable peptidoglycan. In this technique, pre-existing peptidoglycan is labelled with D-cysteine and is detected in isolated sacculi with antibiotin antibody. In a series of pulse-chase experiments, de Pedro *et al.* (1997) expanded previous observations by establishing that the poles of *E. coli* are metabolically inert and segregate conservatively for at least five generations, the boundary between the pole and cylindrical wall being 'remarkably sharp' (de Pedro *et al.*, 1997). Strikingly, rings of inert peptidoglycan were synthesized at potential septation sites in filaments of several temperature sensitive division mutants. Inert hoops appeared in strains carrying mutations affecting FtsA, FtsQ and FtsI (PBP 3), but not in an FtsZ(ts) mutant and not in cells perturbed for DNA replication (de Pedro *et al.*, 1997). Thus, a ring of iPG may represent the earliest visible stage of septal differentiation, and FtsZ, either alone or via unknown proteins, may initiate synthesis of this uniquely situated peptidoglycan (Rothfield, 2003).

iPG and morphological defects

Recently, an important relationship was established between this inert peptidoglycan and morphological abnormalities in aberrantly shaped *E. coli* (de Pedro *et al.*, 2003). As mentioned previously, cells lacking multiple LMW PBPs are extremely abnormal. When the location of iPG was measured in sacculi of one such mutant, distinct patches of inert material appeared at positions where malformed cells exhibited kinks, bends or branches. Even small bulges and irregularities were associated with long-lived segments of iPG (de Pedro *et al.*, 2003). Two observations are especially informative. First, iPG was always associated with the original poles and with 'ectopic' poles of branched cells. Second, in several cases, iPG appeared to survive as partial rings, incomplete arcs or patchy elements in the cylindrical portion of the wall. Moreover, these relatively large-scale aggregates seemed to persist longer in mutants than in the parent strain (de Pedro *et al.*, 2003). A similar phenomenon may occur in *B. subtilis* mutants lacking PBP 1 (Pedersen *et al.*, 1999; McPherson and Popham, 2003). These mutants are thin and bent, and about one-third have abnormal septa or unusually thick, circumscribed deposits of peptidoglycan along the interior surface of their walls. The nature and stability of these deposits is unknown, but they are often associated with morphological alterations and some appear to be remnants of interrupted invagination, implying they were laid down by an aborted FtsZ-dependent process.

The strong correlation of iPG with morphological abnormalities suggests that these inert patches or swaths constitute imperfections in the wall which distort the local geometry of the sacculus. If the iPG fragments are inflexible, they will dictate the shape adopted by new peptidoglycan polymerized in their vicinity. Thus, synthesis of iPG at inappropriate positions would explain the shape anomalies in PBP mutants, and may explain the morphological deficiencies of other mutants, as well.

A shape idea

The framework: inert peptidoglycan

From the above observations, it is but a small step to propose that the regulated synthesis and localization of inert peptidoglycan determines the gross shape of normal cells. By definition, these inert fragments would be relatively impervious to normal recycling mechanisms and would behave as sturdy, long-lived braces or supports. Intracellular turgor pressure would govern the shape of recyclable peptidoglycan (rPG) located between these rigid segments, but overall cell shape would be dictated by the geometrical arrangement of inert swaths or patches.

Establishment: FtsZ localizes iPG synthesis

As discussed earlier, mutations and manipulations that alter the activity or localization of the *E. coli* Z ring often give rise to unusually shaped cells. The data are most easily harmonized by presuming that FtsZ plays an unspecified role in morphology. In view of the tight association between inert peptidoglycan and the morphological discontinuities in misshapen cells, the simplest interpretation is that FtsZ participates in synthesis and localization of iPG. This is probably true during normal cell division because the earliest observable step in septal development seems to be the FtsZ-dependent synthesis of a hoop of iPG (de Pedro *et al.*, 1997). It is reasonable to suppose that if conditions allow FtsZ to polymerize elsewhere, then partial Z rings or incomplete arcs could initiate iPG synthesis at atypical positions. Should they persist, these bits of misplaced or extended iPG may develop into the ectopic poles and inflection points of aberrantly shaped cells. Morphological irregularities would arise from a combination of the frequency of FtsZ polymerization at uncharacteristic sites, the lifetimes of such polymers, and the ability of these structures to activate iPG synthesis.

It must be noted that this emphasis on the role of FtsZ in cellular morphology seems at odds with the conclusions drawn by Gullbrand *et al.* (1999). These authors observed branching at the restrictive temperature in *E. coli* mutants expressing the temperature-sensitive FtsZ84 protein and saw no consistent association of Z rings with

branch points, suggesting that FtsZ plays at best a minor role in this process (Gullbrand *et al.*, 1999). However, their results and the ideas expressed here can be reconciled if nucleation of a branch point and elaboration of a branch are separate events. In this view, transient polymerization of FtsZ84 would initiate synthesis of iPG at inappropriate sites, after which the responsible Z ring (or incomplete Z arc) would disappear to reassemble elsewhere. The extra patch of iPG may then behave as an ectopic pole and continued peptidoglycan synthesis would extend it, creating a branch. Thus, once formed, misplaced iPG would give rise to branches in the absence of active FtsZ84. Just as Gullbrand *et al.* (1999) conclude, aberrant septation *per se* would not be responsible for branching. However, incomplete or inaccurate Z-ring activity would still be the decisive factor that generates iPG, which would constitute the 'previous cell wall abnormalities' that the authors surmise lead to branching (Gullbrand *et al.*, 1999).

Maintenance: scaffolding localizes rPG

Although iPG may determine overall cell shape by providing a framework, other internal or external scaffolds must still guide the shape of newly synthesized peptidoglycan connecting these molecular girders. Synthesis of a uni-

form cylindrical wall certainly depends on the activities of FtsZ, MreB, Mbl and other proteins. The dimensions of a pre-existing wall may influence the exact geometries of these internal scaffolds, which may in turn position the peptidoglycan synthetic apparatus so as to assemble a sacculus of defined shape. Agents external to the wall may act similarly or, as in the case of the flagella of *B. burgdorferi*, may impose an additional tertiary structure (Motaleb *et al.*, 2000).

Controlling iPG synthesis

How might a cell regulate the judicious arrangement of iPG to create a specific shape? Potential mechanisms could: (i) affect the supply or localization of precursors; (ii) dictate the location, rate of formation, quantity, or chemical nature of glycan chains and cross-links; or (iii) modify the location, rate or extent of peptidoglycan degradation (Fig. 3). As described above, FtsZ would drive the second mechanism. The first and third would be mediated by the LMW PBPs.

Substrate availability

The DD-carboxypeptidase activity of PBP 5 removes the

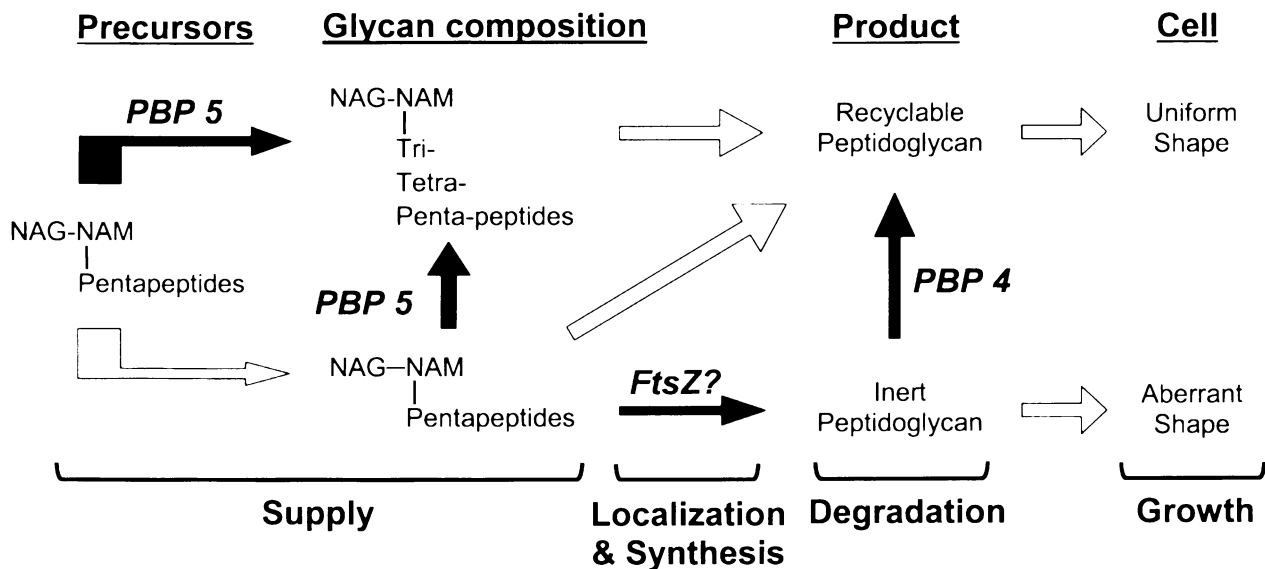


Fig. 3. Hypothetical steps in the synthesis and degradation of inert peptidoglycan in *E. coli*. Muropeptides ('Precursors') are synthesized in the cytoplasm and delivered to synthetic enzymes in the periplasm where they are polymerized and cross-linked ('Glycan composition'). Different enzymes create either recyclable or inert peptidoglycan ('Product'). It is not known whether PBP 5 uses monomer precursors or polymeric peptidoglycan (or both) as substrates, so both alternatives are illustrated ('Supply'). Although PBP 5 is the most active PBP at the steps indicated, other DD-carboxypeptidases (PBP 4, PBP 6 or DacD) may also contribute. The participation of FtsZ ('Localization and Synthesis') is hypothetical and its exact function is unknown. The removal of inert peptidoglycan ('Degradation') is hypothetical, and may be accomplished by PBP 4 (unpublished) or by other hydrolases (endopeptidases, amidases or lytic transglycosylases). Such modification of inert peptidoglycan could create a recyclable form (top product), or the modified components could be degraded and recycled to the cytoplasm (not shown). These degradative enzymes might also act earlier to remove a substrate required by the FtsZ-dependent step. NAG, *N*-acetylglucosamine; NAM, *N*-acetylmuramic acid; PBP, penicillin-binding protein; Pentapeptide, L-al α - γ -D-glu-Dap-D-ala-D-ala; Dap, diaminopimelic acid. Open arrows indicate activity of peptidoglycan synthetic enzymes; closed arrows indicate hypothetical activity of the indicated proteins.

terminal D-alanine from a percentage of pentapeptide side-chains, creating a tetrapeptide that can act only as a recipient during subsequent cross-linking (Fig. 3, 'Supply') (Höltje, 1998). Elimination of PBP 5 increases the concentration of pentapeptides and the pool of available donor side-chains, which must be critical because PBP 5 is the most influential low molecular weight PBP in terms of generating misshapen cells in multiple mutants (Nelson and Young, 2000; Nelson and Young, 2001). Remembering that deformities in such mutants correlate with an increase in iPG (de Pedro *et al.*, 2003), these phenomena are incorporated into the hypothetical scheme of Fig. 3 by proposing that PBP 5 regulates the level of a pentapeptide substrate required for iPG synthesis. Perhaps a similar phenomenon accounts for morphological changes observed in a *B. subtilis* mutant impaired in isoprenoid biosynthesis, which is required for delivery of precursors for peptidoglycan and teichoic acid synthesis (Campbell and Brown, 2002).

Removing excess iPG

We may expect that some superfluous inert peptidoglycan is synthesized no matter what controls its production. An *E. coli* mutant with a high percentage of grossly deformed cells requires deletion of genes encoding at least three PBPs, one of which must be PBP 5 (Nelson and Young, 2001). For example, a strain lacking PBPs 4, 5 and 7 produces extraordinarily irregular cells. PBPs 4 and 7 are endopeptidases that cleave pre-existing cross-links between glycan chains of peptidoglycan. Therefore, a significant morphological effect occurs when at least two different functions are removed from the cell. If, in the absence of PBP 5, PBPs 4 and 7 cleave and remove inadvertently synthesized iPG, the effect of losing PBP 5 may be masked (Fig. 3). Eliminating this degradative pathway in a triple mutant may allow iPG fragments to persist long enough to be incorporated into the wall, creating shape deformities.

Although the above posits that PBPs 4 and 7 remove iPG, any of a number of peptidoglycan hydrolases (endopeptidases, amidases, transglycosylases) might do the same (Höltje, 1998). Of course, the scenario leaves many unanswered questions. Why don't the hydrolases remove *all* inert peptidoglycan, including that synthesized at the site of septation? Is their capacity for iPG removal limited? Might they act only on iPG below a certain size threshold? Is iPG distinguished from rPG by a certain type of cross-linking, or is iPG inert for some other reason but still susceptible to hydrolysis? Is the iPG converted to rPG, or is it degraded to monomers and recycled via the cytoplasm? Fortunately, these and other questions growing out of this proposed scheme can be addressed experimentally.

Concluding remarks

A decade ago Åkerlund *et al.* (1993) suggested that the 'underlying control circuitry and the cell division components of rod-shaped and branching bacteria may be more similar than expected'. In fact, many prokaryotes may share large portions of a common morphogenetic apparatus, suitably adapted to sustain a form selected from the universe of possible shapes. To create any desirable shape a cell need only possess an 'iPG synthesizing apparatus' and be able to control its movement and the activity of the enzymes within it. Of course, the mechanisms restricting enzyme activity to specified areas of the cell await a complete description, but the recent confluence of results should begin to answer long-held questions about bacterial shape.

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Note added in proof

While this review was in press, Shih *et al.* reported that the MinCDE proteins also form distinct spiral structures within the cytoplasm of *E. coli* (Shih, Y.-L., Le, T., and Rothfield, L., 2003, *Proc Natl Acad Sci, USA* online publication 10.1073/pnas.1232225100). The Min spirals differ from those formed by MrcB and arc dynamic, oscillating from pole to pole within the cell. The same considerations apply to these spirals as discussed in the text regarding the use of other proteins as internal scaffolds.

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