Cell shape determination in *Escherichia coli*
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The rigid cell wall peptidoglycan (murein) is a single giant macromolecule whose shape determines the shape of the bacterial cell. Insight into morphogenetic mechanism(s) responsible for determining the shape of the murein sacculus itself has begun to emerge only in recent years. The discovery that MreB and Mbl are cytoskeletal actin homologues that form helical structures extending from pole to pole in rod-shaped cells has opened an exciting new field of microbial cell biology. MreB (in Gram-negative rods) and Mbl (in Gram-positive species) are essential for murein synthesis along the lateral wall and hence, the rod shape of the cell. Known members of the morphogenetic system include MreB (or Mbl), MreC, MreD and PBP2, but Rod A and murein biosynthetic enzymes involved in peptidoglycan precursor synthesis and assembly are likely to be recruited to the same multimolecular apparatus. However, the actual role of MreB in assembly of the morphogenetic complex is still not clear and little is known about regulatory mechanisms controlling the switch from lateral murein elongation to septal murein synthesis at the time of cell division.

**Introduction**

It has been known for almost 50 years that the intact and purified murein (peptidoglycan) sacculus retains the shape and size of the cell from which it has come [1] and that disruption of the structural integrity of the rigid peptidoglycan leads to the loss of normal cell shape. In this sense, murein determines the bacterial cell shape. But what determines the shape of the murein sacculus? As early as 1971, Schwarz and Leutgeb [27] showed that restoration of murein synthesis in *E. coli* spheroplasts gave rise to osmotically resistant round cells whose isolated sacculi were also round. They concluded (1) that sacculus shape was not determined solely by chemical composition or structure of the peptidoglycan polymer, which was indistinguishable by available analytical methods and (2) that there must therefore exist some morphogenic apparatus responsible for determining the shape of the sacculus. The prediction was indeed correct, but it has taken over 30 years to identify major morphogenetic factors and begin to understand their roles in the complex interplay that results in synthesis of a murein sacculus of fixed and reproducible size and shape.

This review focuses primarily on the roles of the cytoskeletal Mre system in determining cell shape in *E. coli* and on possible mechanisms for control of cell diameter. For broader or different perspectives, the reader is referred to several excellent recent reviews [3–6,7*,8*].

**Peptidoglycan biosynthesis and the cell cycle**

Rod-shaped cells face two distinct problems in replicating their shape over the course of the cell cycle. Cell elongation requires lateral extension of the murein sacculus by intercalation of new glycan strands and cross-linking of peptide subunits into the pre-existing shell [9]. However, as the cell prepares for cell division, elongation ceases and the cell switches to specific synthesis of septal peptidoglycan that will form the new pole of each daughter cell.

A distinguishing characteristic of polar murein is its stability *in vivo*. Pulse-chase experiments have shown rapid turnover of lateral murein. By contrast, polar, that is, septal murein is metabolically inert, showing essentially no turnover of glycan or peptide subunits in subsequent generations [10**]. Interestingly, a second type of inert peptidoglycan was revealed in experiments in which septation was prevented. Discrete patches of stable murein were seen in the resulting non-septate filaments at regular intervals that corresponded to potential division sites. This inert ‘preseptal murein’ is of particular interest [11*] and is discussed further in a later section.

**The roles of penicillin binding proteins (PBPs) in murein synthesis and cell shape determination**

Covalent binding by penicillins defines a menagerie of proteins (in *E. coli* no fewer than 12 [12]) that are involved in peptidoglycan metabolism and establishment/maintenance of normal sacculus morphology. The high molecular weight family – PBPs 1a, 1b, 1c, 2, and 3 – are enzymes with transglycosylase and/or transpeptidase activities and catalyze synthesis and insertion of new polymer strands into the sacculus [12]. As such, these are obvious candidates for control by the putative morphogenetic apparatus. The remaining seven, of lower
molecular weight (PBP4-7, DacD, AmpC, AmpH), include amidases and peptidases that cleave either polymeric peptidoglycan or disaccharide–pentapeptide precursor subunits [12].

Mutational studies originally showed that PBP2 and PBP3 carry out different and complementary functions in establishment of the rod shape. PBP2 is required for lateral wall synthesis; loss of function results in cessation of cell elongation and formation of round cells [13]. Conversely, PBP3 is essential for septal murein synthesis; inactivation gives rise to long non-septate filaments [13]. Localization studies using fluorescence techniques have generally re-inforced these functional assignments. Thus, GFP-PBP2 was found in foci along the lateral wall, as expected for its role in cell elongation [14]. More surprisingly, the protein was also concentrated at midcell in septating cells, suggesting a hitherto unrecognized function in cell division. Again consistent with mutational analysis, PBP3 (FtsI) was primarily localized at midcell [15] but appeared diffusely along the cylinder in non-dividing cells [16]. The results suggest the existence of specific morphogenetic switches to account for the redistribution of both PBP2 and PBP3 during the cell cycle.

The PBP1 family of bifunctional transglycosylase–transpeptidases is thought to participate in both lateral and septal murein syntheses. Consistent with this idea, studies on localization of PBP1b showed immunofluorescent staining both along the length of the cell and at division sites [17]. Interestingly, PBP1b interacted directly with PBP3 both in vivo and in vitro, potentially accounting for its divisomal localization.

**Regulation of PBP localization and activity**

**The Mre system and PBP2 function**

Mutations in MreB result in conversion from rod shape to sphere [18], implying a role for MreB in cell shape determination. The discovery by Errington and colleagues [19] that MreB and its homologue in B. subtilis, Mbl, form cytoskeletal structures – extended filamentous helices that underlie the cell membrane – created an explosion of interest in bacterial cytoskeletal elements in general and the function of Mre proteins in determination of rod shape in particular. The extraordinary similarity of the MreB crystal structure to that of eucaryotic actin [20] firmly established it as a true cytoskeletal protein.

Disruption of the pole-to-pole MreB helix by mutational inactivation or exposure to the specific inhibitor, A22, results in failure of lateral murein synthesis and formation of spherical cells [21,22,23], implicating the MreB cytoskeleton in determination of rod shape. The phenotype is similar to that resulting from inactivation of PBP2, suggesting a functional relationship between the two proteins. Disruption in the two genes immediately down-stream of MreB, MreC, and MreD also yielded spherical phenotypes in E. coli and B. subtilis [23,24]. In B. subtilis, Errington and colleagues showed that synthesis of lateral murein is governed by the MreB homologue, Mbl, [25] and that localization of GFP-MreC and GFP-MreD appeared to be similar to that of Mbl [23]. Kruse et al. [24] also showed that E. coli MreC directly interacts with MreB and MreD in a bacterial two hybrid system. In addition, MreC, a bitopic membrane protein, interacts with several high molecular weight PBPs, including PBP2, in affinity chromatography (in C. crescentus [26]) and in a bacterial two hybrid system (in B. subtilis [27]).

These results suggest that lateral murein synthesis is carried out by a helical array of interacting proteins that includes MreB (or Mbl), MreC, MreD, and PBP2. The model predicts that new peptidoglycan is inserted into the lateral wall in a helical pattern that reflects the helical pattern of the biosynthetic complex. Indeed, labeling of nascent peptidoglycan in B. subtilis with fluorescent derivatives of vancomycin [25,28] or ramoplanin [28] showed clear helical distribution along the lateral wall, and it is highly probable that E. coli peptidoglycan is also inserted helically [10,29].

Three possible mechanisms for the roles of the MreB (and Mbl) helices in lateral murein synthesis might be considered: (1) the MreB or Mbl helix might act as a permanent scaffold protein that recruits and maintains a multiprotein assemblage responsible for lateral wall synthesis; (2) MreB (or Mbl) might act as a transient scaffold, analogous to the scaffold proteins required for viral capsid assembly, which disassemble when head formation is complete. MreB would then be required for assembly, but not for maintenance, of the murein biosynthetic apparatus; (3) MreB might not function as the primary scaffold, but be secondarily recruited to another, pre-existing helical scaffold for assembly of the murein biosynthetic complex.

The actual role of the MreB cytoskeleton is still unclear. The helical distribution of MreC and MreD is apparently independent of MreB. In Caulobacter, Dye et al. [21] found that MreC formed spirals that did not colocalize with MreB helices and persisted in cells treated with the MreB inhibitor, A22, under conditions in which MreB helices disassemble. Conversely, MreB helices were retained in cells depleted of MreC. GFP-PBP2 also showed a helical distribution in Caulobacter [30]. The helical distribution depended on MreC, and the helical pattern partially overlapped with MreC but not with MreB. The authors reported (Figure 7B in reference [30]) the PBP2 organization was lost in MreB-depleted cells, but the present authors believe that these images show clear evidence of a helical pattern. In the studies of Kruse et al. [24] in E. coli, the distribution of GFP-MreC also appeared significantly different from that of MreB.
even though the two proteins interacted directly in the two-hybrid system.

Overall, the results to date suggest that MreB and MreCD helices may be independent structures. However, the question of the relationship between MreB, MreCD, and PBP2 helices merits re-examination, especially in round cells of *E. coli* resulting from depletion of one of these. It would be interesting to add spheroplasts to the experimental list to test the possible scaffold function of peptidoglycan. Carballido-Lopez and Errington [31] reported that the helical pattern of Mbl was ‘fragmented’ in *B. subtilis* protoplasts, but immunofluorescence still showed a pattern of peripheral dots that could be part of an organized cytoskeletal arrangement in the round cells. In retrospect, three-dimensional reconstruction might be revealing.

Even if MreB is not itself the primary scaffold for assembly of the biosynthetic machinery, it might function to recruit a limited subset of players to sites of biosynthesis. Indeed, Carballido-Lopez et al. [32] have shown that MreBH, one of the MreB isomorphs in *B. subtilis* that colocalize with MreB, is required for helical organization of the periplasmic lytic endopeptidase, LytE, and interacts with it in the yeast two hybrid system.

In addition to the helical pattern that extends from pole to pole, bands of MreB at or near midcell have been observed consistently in a fraction of the cell population (see reference [7] for review). Time-lapse studies in *Caulobacter* [33] demonstrated shifts in MreB distribution from a pole-to-pole helix during elongation to a midcell band or ring in predivisional cells, providing clear evidence that the distribution of the protein is dynamic over the cell cycle. It is tempting to speculate that the MreB rings may be involved in the formation of preseptal murein, bands of metabolically inert peptidoglycan at or near potential division sites in non-septate filaments of FtsA, FtsQ, or PBP3 (FtsI) mutants of *E. coli* [10]. Interestingly, the bands were absent from FtsZ-depleted filaments.

**RodA and cell shape: still a mystery**

Mutational inactivation of RodA yields an osmotically resistant, round-cell phenotype indistinguishable from that resulting from antibiotic or mutational inactivation of PBP2 (see references [34,35]). De Pedro et al. [34] have provided convincing evidence in *E. coli* that, in both cases, synthesis of new lateral wall ceases and synthesis of septal murein becomes constitutive. The round cells are, in effect, composed of two poles with no sidewall murein in between. Unfortunately, the role of RodA in lateral murein synthesis and its relationship to PBP2 localization and/or activity remain unclear. RodA is highly homologous to FtsW, a component of the divisome that is required to recruit PBP3 to midcell [35] possibly suggesting that RodA may play a similar role in PBP2 localization to lateral wall or midcell or both. To our knowledge, RodA has not been localized, and the question of its interactions with PBP2 and the Mre family remains unclear. It has been suggested [36] that RodA and FtsW may be involved in recruitment of lipid-linked murein precursors to sites of synthesis.

**Control of cell width: another mystery**

The cell width of a given *E. coli* strain is remarkably constant during steady-state exponential growth. However, changes in growth rate result in significant changes in cell diameter, such that rapidly growing cells are relatively fat and slowly growing cells are relatively thin. The mechanisms regulating cell width remain almost entirely unknown. Growth rate information must somehow be translated into the altered size of the murein sacculus, but how is the rate change sensed by the machinery for wall growth? And how can that machinery respond to change the diameter of the rod cylinder?

It has been proposed [37] that a change in growth rate may be sensed as a change in cytoplasmic turgor pressure, hence a change in the stress placed on the cell envelope. The stress theory of morphogenesis is discussed in detail by Harold in this issue. It should be emphasized, however, that there are other possible transduction mechanisms, for example, changes in concentrations of second messengers or other signaling molecules (see reference [38] for an example) that might be recognized by the cytoskeleton and murein synthetic machinery.

Whatever the signaling mechanism, the response to a change in growth rate may involve changes in the formation or maintenance of inert murein. Murein at cell poles is normally inert, neither undergoing new synthesis nor autolytic turnover, and nascent septal murein has been shown to mature to the inert state very rapidly after synthesis [39]. It is possible that the diameter of the cylinder is established during cell division when the geometry of the septum (new cell pole) is determined. Then how is the final diameter of the new pole established at cell division? Preseptal murein, which is formed at or adjacent to potential division sites is also metabolically inert and could play a role in establishing the diameter of the nascent new pole during septation. The reader is referred to the 2003 review by Young [3] for an excellent discussion of inert murein.

A question that has apparently not yet been addressed experimentally asks what happens during the transition to the new cell diameter as a result of changing growth rate, or during reversible rod-to-sphere transitions resulting from inactivation or re-activation of lateral murein synthesis. At early stages of the rod-to-sphere transition, pear-shaped cells are seen in which the old pole retains...
Outlook

Despite significant recent progress, an understanding of how rod-shaped bacteria such as *E. coli* establish, maintain, and modify cell shape remains elusive. We know that enzymes responsible for synthesis of murein along the length of the cell cylinder are associated with cytoskeletal elements that are likely to play important roles in the topology of new cell wall synthesis. We also know that the Mre cytoskeleton is dynamic, but we understand little of the how or why. We speculate that the cytoskeleton may act to transduce cellular signals to regulate synthesis of cylindrical murein and/or to regulate the dimensions of the cell cylinder, but information remains lacking.

We are confident that our understanding of cell shape control will increase rapidly. Major immediate challenges will be to understand the molecular mechanisms responsible for cell shape changes under different growth conditions, and to define the biological significance of the cell cycle related shifts in cellular location of cytoskeletal proteins and murein biosynthetic proteins. It is not unlikely that significant surprises await us.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


A thorough and up-to-date discussion of cytoskeletal structures and functions in bacteria.


First demonstration that polar murein is inert.


First suggestion that FtsZ may play a role in prespore murein synthesis.


First raised possibility that distribution of murein biosynthetic enzymes was dynamic over the cell cycle.


The initial demonstration, together with reference [25], that rod-shaped cells have extended cytoskeletal structures that play a role in cell shape determination.


33. evidence in B. subtilis that cytoskeletal elements act to recruit enzymes involved in murein morphogenesis.


36. Demonstrates that RodA is essential for rod shape in B. subtilis.


43. Shows that PBPS mutants accumulate patches of inert murein that are responsible for a branching cell phenotype.