

Rotate into shape: MreB and bacterial morphogenesis

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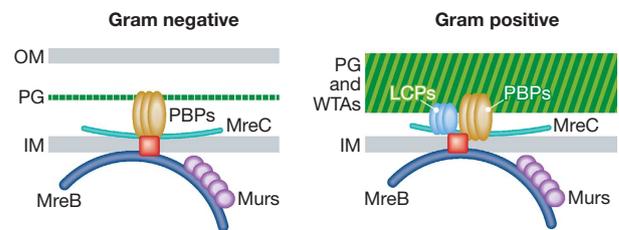
MreB, the bacterial actin homologue, plays a vital role in determining cell shape, but the mechanisms by which it actually functions have remained largely mysterious. Recent studies now shed new light on MreB, demonstrating that it associates with many cell-wall synthesis enzymes, including a newly identified family of proteins that mediate teichoic acid synthesis in Gram-positive bacteria. Furthermore, MreB filaments dynamically rotate around the cell circumference in a manner dependent on the cell-wall assembly machinery. Thus, MreB may function to spatially organize the enzymatic activities required for proper bacterial growth (see Figure 1).

In nearly all bacteria, cell shape is determined by the structure of the cell wall, a rigid crosslinked meshwork that counteracts the cellular turgor pressure. Bacteria can be generally separated into Gram-negative species that have a thin peptidoglycan (PG) cell wall and both an inner and outer membrane, and Gram-positive species with only one membrane and a thick cell wall composed of both PG and wall teichoic acids (WTAs). In both groups, PG biosynthesis is carried out by a series of Mur enzymes that synthesize PG precursors in the cytoplasm and several penicillin binding proteins (PBPs) that polymerize and crosslink these subunits once they are exported (Vollmer and Seligman, 2010).

Given that both MreB and the various PG and WTA enzymes are required for proper growth, the challenge has been to develop a biophysical understanding of the interaction between these two systems. An important step forward was recently made in three independent papers that link the dynamics of MreB to cell-wall synthesis in two evolutionary distinct organisms, the Gram-negative bacterium *Escherichia coli* (van Teeffelen *et al.*, 2011) and the Gram-positive *Bacillus subtilis* (Dominguez-Escobar *et al.*, 2011; Garner *et al.*, 2011). All three studies found that MreB polymers rotate around the long axis of the bacterial cell on a time scale of 1–5 min. While MreB motion had previously been attributed to its polymerization, these new studies show that this rotation is not caused by polymerization but rather depends on cell-wall synthesis activity. Specifically, if cell-wall precursors are depleted or cell-wall synthesis enzymes inhibited with antibiotic drugs, then MreB rotation stops. In *B. subtilis*, such rotational motion was observed for the PG transpeptidases PbpH and Pbp2A as well as for the MreB-associated proteins MreC, MreD, and RodA, which are believed to be part of cell-

wall synthesis complexes. In the *E. coli* system, analysis of MreB dynamics supported a quantitative model in which processive cell-wall assembly enzymes are physically linked to MreB such that PG synthesis pulls MreB around the cell

A Recruitment of cell-wall synthesis enzymes



B Spatio-temporal control of PG/WTA insertion

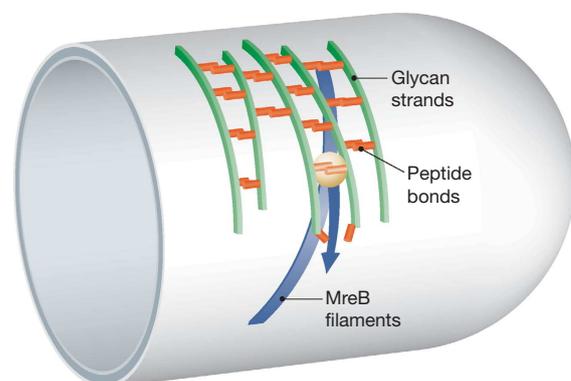


Figure 1 Potential functions of the MreB cytoskeleton in regulating cell shape. **(A)** MreB (dark blue) recruits several classes of enzymes involved in cell-wall synthesis to the sites of peptidoglycan (PG) and wall teichoic acids (WTAs) insertion. In many Gram-negative (left) and Gram-positive (right) bacteria, these enzymes include the PG assembly PBP enzymes (brown), the PG-precursor synthesizing Mur enzymes (purple), MreC (green), and other transmembrane proteins involved in cell elongation such as RodA, RodZ, and MreD (red). The WTA synthesizing LytR–Cps2A–Psr (LCP) enzymes (blue) also associate with MreB in Gram positives. **(B)** By restricting insertion of new glycan strands (green) and peptide bonds (red) to sites close to MreB filaments (magenta), the cell might robustly maintain rod-like shape during growth.

(van Teeffelen *et al*, 2011). Together, the three studies indicate that cell-wall synthesis enzymes and MreB are tightly coupled and move circumferentially around the long axis of the cell while inserting new PG material. It remains unclear whether the energy for MreB rotation is directly powered by cell-wall synthesis or whether cell-wall synthesis is indirectly required for an as-yet unidentified motor protein.

In addition, two other recent publications use different approaches to demonstrate that MreB may coordinate the activity of a wide range of enzymes involved in bacterial growth (White *et al*, 2010; Kawai *et al*, 2011). White *et al* demonstrated that in the Gram-negative bacterium, *Caulobacter crescentus*, MreB also coordinates the localization of at least some of the cytoplasmic Mur proteins. Notably, the second study, published in this issue of *EMBO Journal*, extends the reach of MreB beyond PG to WTA assembly in *B. subtilis* (Kawai *et al*, 2011). Kawai *et al* used a co-immunoprecipitation assay to identify proteins that interact with any of the three *B. subtilis* MreB isoforms, MreB, Mbl, and MreBH. By crosslinking, affinity co-purification and liquid chromatography/mass spectroscopy they found >100 MreB-interacting proteins including the PG synthases already known to interact with MreB, thus validating their approach. MreB also interacted with a number of known Tag proteins that have been previously implicated in WTA synthesis. Given the association with the known Tag proteins, the authors set out to determine if the MreB-interacting proteins might also include the previously unidentified transferases that connect WTAs to the PG network. They focused on three uncharacterized proteins that had significant extracellular domains and were linked to known tag gene operons. These proteins, newly named TagT (previously known as YwtF), TagU (LytR), and TagV (YvhJ), represent members of a widely distributed class of LytR-Cps2A-Psr (LCP) proteins found in all Gram-positive bacteria that contain anionic polymers. As predicted, a strain with a triple deletion of *tagTUV* was unable to synthesize WTAs. The authors went on to crystallize the soluble domain of the Cps2A LCP homologue from *Streptococcus pneumoniae*. Interestingly, the protein was found to bind phosphorylated polyisoprenoid lipids, suggesting that LCPs may indeed mediate the elusive phosphotransfer reaction. Biochemistry and mutagenesis studies further supported this hypothesis, although the actual reaction with WTAs could not be recon-

stituted *in vitro*. Importantly, both TagT and TagU showed a similar localization pattern to MreB and became delocalized in *mreB mbl* double mutants. Together, these findings both identify a novel WTA activity and support the hypothesis that MreB is an important organizer of multiple aspects of cell-wall synthesis.

Given that MreB is coupled to the cell-wall synthesis machinery, what is the function of such coupling? One possibility is that MreB provides a scaffold for cell-wall synthesis enzymes and cytoplasmic enzymes required for building cell-wall precursors. By associating with the same MreB backbone, these enzymes would be able to interact in a coordinated fashion and cell-wall precursors could efficiently be used at the location of their synthesis (Figure 1A). The *B. subtilis* studies support this view: the cell-wall synthesis components Pbp2A (Garner *et al*, 2011), RodA, and PbpH (Dominguez-Escobar *et al*, 2011) move non-processively and in random orientations in the absence of MreB. However, *E. coli* cells can grow in the complete absence of MreB (Bendezu and de Boer, 2008), suggesting that while MreB may enhance the efficiency of PG synthesis, it is not absolutely required. An alternative but not mutually exclusive possibility is that MreB spatially determines or constrains the potential sites of cell-wall synthesis, as suggested by Furchtgott *et al* (2011) and van Teeffelen *et al* (2011) (Figure 1B). Computational simulations of growing cell-wall sacculi suggest that helical and stiff MreB polymers could facilitate the stability of rod-like cell shape in Gram-negative bacteria by enforcing a uniform density of PG insertion events in the cell wall. This mechanism relies on the stiffness of MreB filaments that bridge small spatial fluctuations in the PG meshwork. While insertion of new material within this model is still guided by the local orientation of existing, neighbouring glycan strands, the stiffness of MreB renders the stochastic insertion events independent of the local amount of PG. How the morphology of MreB polymers affects the structure of the cell-wall sacculus in this model is currently under investigation. Nevertheless, these recent studies now provide a framework for ultimately deciphering the long-standing mystery of how MreB regulates bacterial cell shape.

Conflict of interest

The authors declare that they have no conflict of interest.

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