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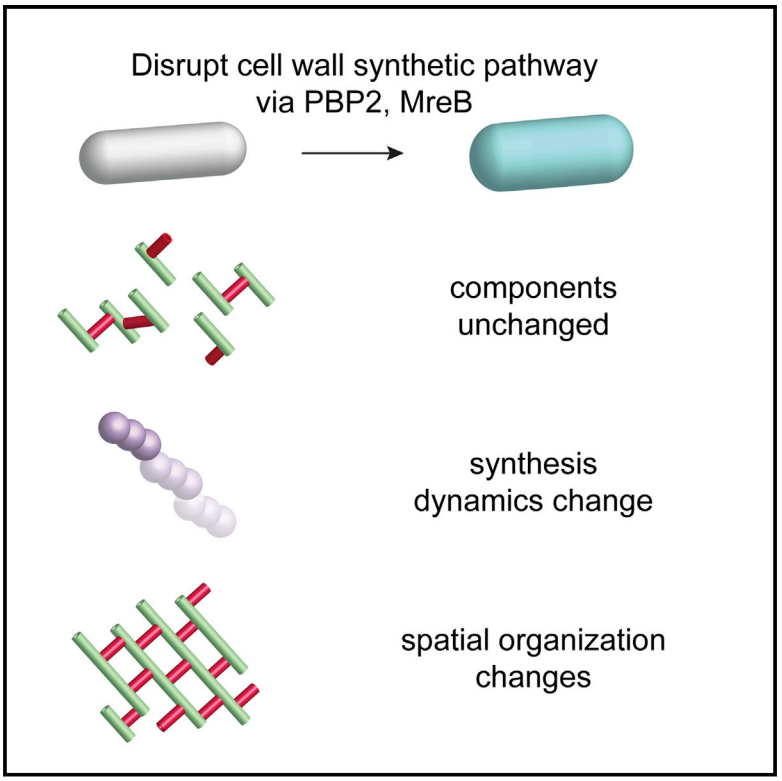
Principles of Bacterial Cell-Size Determination Revealed by Cell-Wall Synthesis Perturbations

Graphical Abstract



Highlights

Heterologous expression of cell-wall enzymes complements growth and alters cell width

Cell-wall enzyme perturbation does not affect chemical composition of peptidoglycan

Orientation of MreB motion and cell twisting are correlated with cell width

Quantification of growth behaviors reveals different roles for MreB and PBP2

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In Brief

Tropini et al. demonstrate genetic and chemical means of modulating *E. coli* cell size. They find that wider cells exhibit systematic large-scale changes in cell mechanics consistent with altered chirality and a more isotropic cell wall. Their results highlight the robustness of cell-wall synthesis and physical principles dictating cell-size control.

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Principles of Bacterial Cell-Size Determination Revealed by Cell-Wall Synthesis Perturbations

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SUMMARY

Although bacterial cell morphology is tightly controlled, the principles of size regulation remain elusive. In *Escherichia coli*, perturbation of cell-wall synthesis often results in similar morphologies, making it difficult to deconvolve the complex genotype-phenotype relationships underlying morphogenesis. Here we modulated cell width through heterologous expression of sequences encoding the essential enzyme PBP2 and through sublethal treatments with drugs that inhibit PBP2 and the MreB cytoskeleton. We quantified the biochemical and biophysical properties of the cell wall across a wide range of cell sizes. We find that, although cell-wall chemical composition is unaltered, MreB dynamics, cell twisting, and cellular mechanics exhibit systematic large-scale changes consistent with altered chirality and a more isotropic cell wall. This multiscale analysis enabled identification of distinct roles for MreB and PBP2, despite having similar morphological effects when depleted. Altogether, our results highlight the robustness of cell-wall synthesis and physical principles dictating cell-size control.

INTRODUCTION

The molecular, chemical, and physical mechanisms that control cell shape have been longstanding questions in all kingdoms of life. In bacteria, cell morphology affects many behaviors, such as cell division, motility, nutrient uptake, and biofilm formation (Justice et al., 2008; Young, 2006). Different species adopt a diverse set of morphologies (Young, 2006), although most species can robustly maintain a particular shape. Elucidating the perturbations that adjust morphology and the biophysical mechanisms that transduce these changes to the cellular scale is critically important for our understanding of bacterial physiology.

Bacterial cell shape is conferred by the peptidoglycan (PG) cell wall, a macromolecular polymer network surrounding the cytoplasmic membrane (Schleifer and Kandler, 1972) that is composed of repeating sugar (glycan) subunits crosslinked by short peptides. In Gram-negative bacteria such as *E. coli*, the cell wall is a predominantly single-layered, dynamic meshwork that maintains an approximately constant width as the cell elongates (Scheffers and Pinho, 2005). A major class of proteins involved in the insertion of new PG is the penicillin binding proteins (PBPs), many of whose biochemical activities (transpeptidation, transglycosylation, hydrolysis) have been characterized using liquid chromatography (Banzhaf et al., 2012; Popham and Young, 2003; Vollmer and Bertsche, 2008). Disrupting the function of the PBPs can cause morphologies such as filamentous, coccal, or branched cells (Popham and Young, 2003).

Spatiotemporal coordination of the PBPs has been linked to the cytoskeletal protein MreB, a homolog of eukaryotic actin that polymerizes into filaments that are colocalized with sites of growth (Ursell et al., 2014; White et al., 2010). Depletion of MreB (Carballido-López, 2006; Wachi et al., 1987) or inhibition of MreB polymerization by the small molecule A22 results in progressive cell rounding and eventual lysis (Bean et al., 2009). The recently discovered cell twisting during *E. coli* growth is MreB dependent and is thought to result from chiral ordering of the PG in which the glycan strands have a right-handed orientation bias (Wang et al., 2012). In both *E. coli* and the Gram-positive rod-shaped bacterium *Bacillus subtilis*, MreB moves circumferentially in a directed manner dependent on cell-wall synthesis (Domínguez-Escobar et al., 2011; Garner et al., 2011; van Teeffelen et al., 2011). MreB motion in *E. coli* is reduced by the addition of mecillinam (Lee et al., 2014; van Teeffelen et al., 2011), a beta-lactam antibiotic that specifically inhibits PBP2, an essential transpeptidase encoded by the gene *mrdA* that participates in glycan strand crosslinking. Depletion of wild-type PBP2 causes cell rounding and eventual lysis, similar to MreB depletion (Lee et al., 2014). Given the similar effects of PBP2 and MreB perturbation, these two proteins are often assumed to work in a conserved linear pathway despite the lack of direct evidence (Osborn and Rothfield, 2007).

Even with the identification of many genes and biochemical activities required for cell-wall synthesis, it has been challenging to uncover the principles that unify related mechanisms of cell-shape maintenance and cell-size determination. Since