Helical insertion of peptidoglycan produces chiral ordering of the bacterial cell wall

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AUTHOR SUMMARY

Cells from all kingdoms of life face the task of constructing a specific, mechanically robust three-dimensional (3D) cell shape from molecular-scale components. For many bacteria, maintaining a rigid, rod-like shape facilitates a diverse range of behaviors including swimming motility, detection of chemical gradients, and nutrient access and waste evacuation in biofilms. The static shape of a bacterial cell is usually defined by the cell wall, a macromolecular polymer network composed of glycan strands crosslinked by peptides. Although the biochemical pathway controlling cell-wall synthesis has been intensively studied, how synthase enzymes are coordinated in space and time to produce a mechanically robust cell wall is poorly understood. We show that a helical cytoskeleton, of the bacterial actin MreB drives the emergence of a uniformly rigid wall with chirally ordered glycan strands. This organization leads to twisting of the cell body of rod-shaped bacteria during elongation.

In this work, we show that the cell-shape determinant MreB, a homologue of the eukaryotic cytoskeletal protein actin, is distributed preferentially as left-handed, helical segments in rod-shaped *Escherichia coli* cells. By combining 3D-imaging techniques with an analysis of the circumferential fluorescence pattern generated by MreB fused to YFP, we precisely determine the helical orientation of MreB in live cells. We then track the motion of beads attached to the top of growing cells and find that cells twist left-handedly during elongational growth (Fig. P1). Previous work on spiral mutants of rod-shaped bacteria has also noted a conserved handedness, though the molecular mechanism for this emergent chirality has remained unexplained.

To link our observations of left-handed MreB distribution and left-handed twisting, we used a biophysical model of cell-wall growth to demonstrate that patterning of cell-wall synthesis by left-handed MreB polymers leads to a right-handed chiral organization of the glycan strands. This organization produces a left-handed twisting of the cell body during elongational growth. We then confirm the existence of right-handed glycan organization in *E. coli* by osmotically shocking surface-labeled cells and directly measuring the difference in stiffness between the longitudinal and transverse directions. The Gram-positive, rod-shaped bacterium *Bacillus subtilis* has a much thicker cell wall and is evolutionarily distant from *E. coli*, yet *B. subtilis* also twists during elongation, albeit with opposite handedness to that of *E. coli*. Taken together, these results link the molecular details of cytoskeletal conformation to global phenomena of cell-surface twisting.

We propose that the previously underappreciated phenomena of elongation-based twisting and the mechanistically coupled chiral organization of the cell wall may be common among rod-shaped bacteria. Furthermore, similar chiral motion during growth has been observed in organisms as diverse as the plant *Arabidopsis thaliana*, the fruit fly *Drosophila melanogaster*, and the African clawed frog *Xenopus laevis* (1–3), raising the possibility that a chiral bacterial cytoskeleton may have served as the evolutionary template for cellular and organismal chirality in other kingdoms.

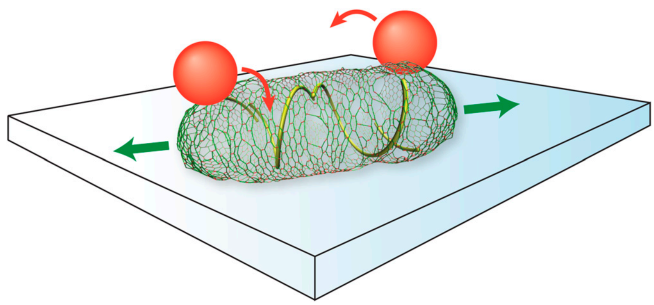

Fig. P1. Helical insertion of material into the bacterial cell wall (green) during elongational growth, guided by the protein MreB (yellow), leads to an emergent chiral order in the cell-wall network and twisting of the cell that can be visualized using surface-bound beads (red).

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Author contributions: S.W., L.F., K.C.H., and J.W.S. designed research; S.W. and L.F. performed research; S.W., L.F., K.C.H., and J.W.S. analyzed data; and S.W., L.F., K.C.H., and J.W.S. wrote the paper.

The authors declare no conflict of interest.

This Direct Submission article had a prearranged editor.

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Cite this Author Summary as: PNAS 10.1073/pnas.1117132109.