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### **Spatial Patterning of Newly-Inserted Material during Bacterial Cell Growth<sup>1</sup>**

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In the life cycle of a bacterium, rudimentary microscopy demonstrates that cell growth and elongation are essential characteristics of cellular reproduction. The peptidoglycan cell wall is the main load-bearing structure that determines both cell shape and overall size. However, simple imaging of cellular growth gives no indication of the spatial patterning nor mechanism by which material is being incorporated into the pre-existing cell wall. We employ a combination of high-resolution pulse-chase fluorescence microscopy, 3D computational microscopy, and detailed mechanistic simulations to explore how spatial patterning results in uniform growth and maintenance of cell shape. We show that growth is happening in discrete bursts randomly distributed over the cell surface, with a well-defined mean size and average rate. We further use these techniques to explore the effects of division and cell wall disrupting antibiotics, like cephalexin and A22, respectively, on the patterning of cell wall growth in *E. coli*. Finally, we explore the spatial correlation between presence of the bacterial actin-like cytoskeletal protein, MreB, and local cell wall growth. Together these techniques form a powerful method for exploring the detailed dynamics and involvement of antibiotics and cell wall-associated proteins in bacterial cell growth.

In collaboration with Kerwyn Huang, Stanford University.

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