BACTERIAL PHYSIOLOGY

Diffusion barrier segments the stalk

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The polar stalk of *Caulobacter crescentus* consists of a cell envelope surrounding a thin cytoplasmic core. At the tip of the stalk is the holdfast, an adhesive organelle that mediates permanent surface attachment, and at irregular intervals along the length of the stalk are cross-bands, which are disk-like structures that span the entire stalk width. The precise composition and function of these cross-bands have remained elusive, but Schlimpert *et al.* now show that they are composed of at least four proteins and act as diffusion barriers for both soluble and membrane proteins.

When *C. crescentus* is grown in phosphate-limiting conditions, the stalk becomes elongated, increasing the surface-area-to-volume ratio of the cell in a manner that is thought to enhance phosphate scavenging. To investigate this phenomenon, Schlimpert *et al.* used fluorescence loss in photobleaching (FLIP) and fluorescence recovery after photobleaching (FRAP) to monitor the mobility of the periplasmic phosphate-binding protein PstS fused to an mCherry fluorescent reporter. They found that when a laser pulse was applied to the cell pole opposite the stalk, the PstS–mCherry fluorescence signal was irreversibly lost throughout the cell body but not from the stalk. Conversely, when the laser pulse was applied to the stalk, there was irreversible bleaching in the stalk but not in the cell body, suggesting that a bidirectional diffusion barrier exists that separates the stalk and the cell body.

To identify the components responsible for this diffusion barrier, the authors screened uncharacterized ORFs that were transcriptionally upregulated at the onset of stalk

formation. Candidate proteins encoded by these ORFs were fused to mCherry, ectopically expressed and assessed microscopically for a potential role in the stalk. Two proteins were observed to form distinct foci that were distributed at irregular intervals along the length of the stalk, and the authors named these proteins stalk protein A (StpA) and StpB. Immunoprecipitation and localization experiments revealed that StpA and StpB formed a complex that also contained two other proteins, which the authors designated StpC and StpD. Three of the proteins (StpA, StpC and StpD) were found to be membrane associated, whereas StpB was soluble but tethered to the membrane in an StpA-dependent manner. Importantly, the authors found that in an *stpAB* mutant, cross-bands were undetectable using electron cryotomography (ECT), despite stalk length and morphology being unaffected. Furthermore, correlated light microscopy and ECT verified that all four Stp proteins were always found at the site of cross-band formation, suggesting that these four proteins are indeed cross-band components.

To examine whether these Stp proteins are important for the diffusion barrier, the authors monitored the mobility of a soluble periplasmic RFP (TAT–tdimer2) in wild-type and *stpAB*-mutant cells using FLIP. When wild-type cells were laser-pulsed at the pole opposite the stalk, TAT–tdimer2 bleaching mostly extended through the cell body but not past the first cross-band in the stalk (although in 20% of cells, bleaching continued to the second cross-band, possibly owing to incomplete assembly of the first cross-band). By contrast, in *stpAB*-mutant cells, TAT–tdimer2 bleaching

continued throughout the stalk. Importantly, similar results were obtained with fluorescent reporters fused to inner-membrane and outer-membrane proteins and even to cytoplasmic proteins.

Finally, the authors sought to investigate the physiological function of the diffusion barrier. They reasoned that because the cross-bands would retain newly synthesized envelope components in the cell body, effectively limiting the size of the active cell envelope, this might provide a competitive advantage during growth in phosphate-limiting conditions, when the stalk would account for most of the periplasmic volume and surface area of the inner and outer membranes. To test this idea, they carried out competitive growth assays and observed that on recovery from phosphate starvation, wild-type cells outcompeted *stpAB*-mutant cells, despite having equal growth rates under these conditions. Furthermore, even in phosphate-rich medium, when stalks are short, wild-type cells had a small but significant growth advantage over *stpAB*-mutant cells.

Taken together, these findings identify the four Stp proteins as components of the cross-bands and show that these proteins function as a diffusion barrier. This barrier allows *C. crescentus* to create a long cellular extension with a specialized function without the expense of having to synthesize the additional envelope components that would be needed if free diffusion were possible.

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ORIGINAL RESEARCH PAPER Schlimpert, S. *et al.* General protein diffusion barriers create compartments within bacterial cells. *Cell* **151**, 1270–1282 (2012)