Bacterial stalks are nutrient-scavenging antennas

Harley H. McAdams*

Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA 94305

Environmental inorganic phosphorus is frequently the growth-limiting nutrient for aquatic microorganisms, and there is fierce interspecies competition for the available supply. What are the limiting factors in effective bacterial scavenging of environmental phosphorus? It depends on the concentration. When the supply is relatively high, the limit is determined by the number of phosphate transporters on the surface times their individual throughput. At very low concentrations, however, the physics of diffusion at small scales comes into play. A relatively sparse distribution of transporters on the cell surface can capture every molecule that approaches the surface and phosphate influx becomes diffusion-limited. The prosthecate (stalked) bacteria, such as *Caulobacter crescentus*, have evolved an effective fitness strategy for these low-phosphorus environments that are dissected by Wagner *et al.* (1) in this issue of PNAS. Long, slender stalks extend the effective surface area of the cell, acting as a sort of nutrient-collecting antenna while adding little to the cell volume (Fig. 1). Thus, the cell's phosphate collection effectiveness is greatly increased at low cost. This hypothesis for the stalk's function was suggested by observations 40 years ago that the prosthecate bacteria grow much longer stalks in phosphate-limited media (2, 3).

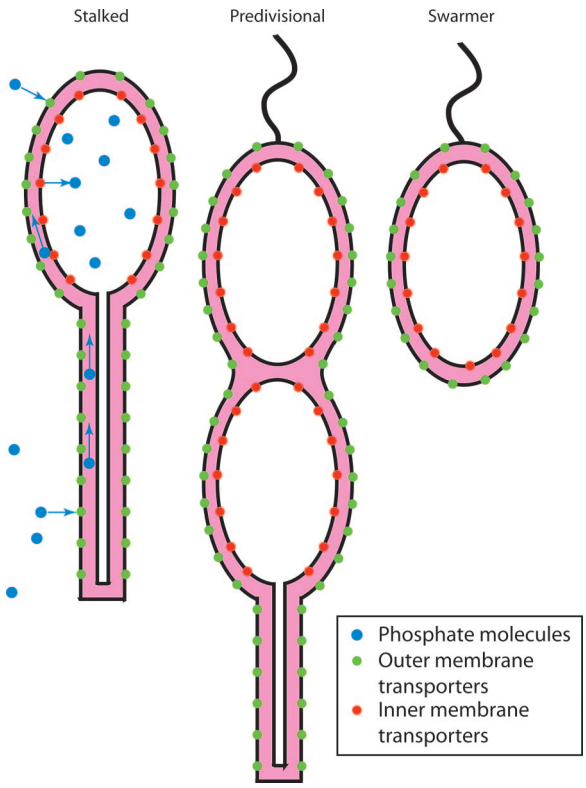

Fig. 1. Phosphate transporters are distributed over the entire *Caulobacter* OM. (*Left*) Phosphate transporters for the IM are not present on the stalk, so that phosphate molecules collected by the stalk must diffuse through the stalk periplasm to the cell body. (*Center*) During cytokinesis, the *C. crescentus* cytoplasm compartmentalizes well before cell separation so that diffusion between the two cytoplasmic compartments is blocked. However, both compartments can benefit from the additional phosphate collected by the stalk and transported through the periplasm. (*Right*) After daughter cell separation, the stalkless swarmer cell will be less effective in phosphate collection in phosphate-limited situations because of its much smaller effective surface area; thus it is more likely than its stalked sibling to be phosphate starved.

Wagner *et al.* (1) use mathematical modeling of the diffusive transport for different cell geometries to confirm the effectiveness of longer stalks in the scavenging of phosphate. They also describe somewhat surprising observations regarding the spatial distribution of inner membrane (IM) and outer membrane (OM) phosphate transporters (1). Phosphate ions captured by transporters on the stalk OM are brought into the periplasm and then must diffuse through the stalk periplasm to the cell body because no phosphate transporters are found along the stalk IM (Fig. 1). Phosphate transporters on the IM of the main cell body then move the phosphate into the cytoplasm.

Transporters on both the stalk and the cell body OMs can import and hydrolyze fluorescein diphosphate (FDP) (1). The fluorescein liberated by hydrolysis of FDP in the periplasm enables a fluorescent microscopy assay of phosphate uptake. Periplasmic phosphate is transported into the *C. crescentus* cytoplasm through the IM by using the high-affinity phosphate ATP-binding cassette transporter (PstSCAB), composed of a high-affinity periplasmic phosphate binding protein (PstS), two IM channel proteins (PstA and PstC), and a cytoplasmic traffic ATPase (PstB) (4–6). Fluorescent microscopy, biochemistry, and two-dimensional liquid chromatography tandem mass spectrometry show that the protein composition of the stalk differs from that of the cell body (1). In stalk fractions, OM and periplasmic proteins, such as the nutrient-binding proteins PstS, PhnD, and PotF, are more abundant than IM and cytoplasmic proteins. The higher levels of PstS, PhnD, and PotF in the stalk and the absence of IM proteins such as PstA and ExbB suggest diffusive transport of stalk-collected phosphate via the stalk periplasm to the cell body periplasm and then transport through the IM of the cell body into the cytoplasm.

How does the differential protein content of the stalk and the cell body arise? One hypothesis is that the IM transporters are localized to specific structures in the cell body IM so they cannot diffuse in the membrane into the stalk. Interestingly, fluorescent-tagged PstA and ExbB exhibit a helical localization pattern (1). Similar helical patterns have been observed for peptidoglycan incorporation (7), OM protein incorporation (8), the cytoplasmic actin-like MreB structures (9–11), the penicillin-binding protein Pbp2 (10, 12), and the periplasmic MreC structure that con-

Conflict of interest statement: No conflicts declared.

See companion article on page 11772.

*E-mail: hmcadams@stanford.edu.

trols cell shape (12, 13). This similarity in spatial distribution suggests that the transporters could be associated somehow with one of these other systems.

C. crescentus divides asymmetrically, producing one motile stalkless swarmer daughter cell and a stalked sibling (Fig. 1). The swarmer cell spends ≈30 min (≈22%) of its cell cycle in this stalkless state. In phosphate-limited environments, the swarmer cell with its much smaller surface area will be more phosphate-restricted than stalked cells. In the late stages of cytokinesis, for ≈17 min before cell separation, the cytoplasm is compartmentalized (Fig. 1 *Center*) (14). The design of the stalk phosphate collection system so that phosphate is delivered to the cell body via the stalk periplasm ensures that the nascent swarmer cell compartment will benefit from the stalk's phosphate scavenging capability. If the phosphate captured by the stalk were immediately passed through the stalk IM and then into only the nascent stalked cell compartment, it would exacerbate the relative phosphate-restricted condition of the swarmer cells.

The *C. crescentus* stalk has proven to be interesting on many levels. The physical design of the cell morphology and the spatial distribution of transporters on the cell's IM and OM are optimized for nutrient collection effectiveness in very low nutrient conditions. Furthermore, developmental controls are present that make longer stalks just when they are needed (6). This is an excellent example where analysis of a complex biological subsystem has required the collaboration of many disciplines, in this case, physicists, biochemists, developmental biologists, and microbial ecologists.

Finally, take note that this "little" problem relating to how stalked bacteria acquire phosphorus is of global ecological significance. The prosthecate bacteria are ubiquitous in all of the Earth's aquatic environments. Phosphorus is a limiting nutrient in determining the productivity of lakes and oceans, and the stalked bacteria, something like 10^{27} or 10^{28} of them on the globe, are central players in scavenging phosphorus in oceans and lakes and reintroducing it into the food chain.

1. Wagner, J. K., Setayeshgar, S., Sharon, L. A., Reilly, J. P. & Brun, Y. V. (2006) *Proc. Natl. Acad. Sci. USA* **103,** 11772–11777.
2. Schmidt, J. M. & Stanier, R. Y. (1966) *J. Cell Biol.* **28,** 423–436.
3. Poindexter, J. S. (1964) *Bacteriol. Rev.* **28,** 231–295.
4. Nikata, T., Sakai, Y., Shibat, K., Kato, J., Kuroda, A. & Ohtake, H. (1996) *Mol. Gen. Genet.* **250,** 692–698.
5. Tamber, S. & Hancock, R. E. (2003) *Front. Biosci.* **8,** s472–s483.
6. Gonin, M., Quardokus, E. M., O'Donnol, D., Maddock, J. & Brun, Y. V. (2000) *J. Bacteriol.* **182,** 337–347.
7. Daniel, R. A. & Errington, J. (2003) *Cell* **113,** 767–776.
8. Ghosh, A. S. & Young, K. D. (2005) *J. Bacteriol.* **187,** 1913–1922.
9. Gitai, Z., Dye, N. & Shapiro, L. (2004) *Proc. Natl. Acad. Sci. USA* **101,** 8643–8648.
10. Figge, R. M., Divakaruni, A. V. & Gober, J. W. (2004) *Mol. Microbiol.* **51,** 1321–1332.
11. Kruse, T., Moller-Jensen, J., Lobner-Olesen, A. & Gerdes, K. (2003) *EMBO J.* **22,** 5283–5292.
12. Dye, N. A., Pincus, Z., Theriot, J. A., Shapiro, L. & Gitai, Z. (2005) *Proc. Natl. Acad. Sci. USA* **102,** 18608–18613.
13. Divakaruni, A. V., Loo, R. R., Xie, Y., Loo, J. A. & Gober, J. W. (2005) *Proc. Natl. Acad. Sci. USA* **102,** 18602–18607.
14. Judd, E. M., Comolli, L. R., Chen, J. C., Downing, K. H., Moerner, W. E. & McAdams, H. H. (2005) *J. Bacteriol.* **187,** 6874–6882.