A nutrient uptake role for bacterial cell envelope extensions

Jennifer K. Wagner†, Sima Setayeshgar‡, Laura A. Sharon§, James P. Reilly¶, and Yves V. Brun**

Departments of *Biology, ‡Physics, and §Chemistry, Indiana University, Bloomington, IN 47405

Edited by A. Dale Kaiser, Stanford University School of Medicine, Stanford, CA, and approved June 8, 2006 (received for review March 13, 2006)

Bacteria exist in a variety of morphologies, but the relationship between cellular forms and biological functions remains poorly understood. We show that stalks (prosthecae), cylindrical extensions of the Caulobacter crescentus cell envelope, can take up and hydrolyze organic phosphate molecules and contain the high-affinity phosphate-binding protein PstS, but not PstA, a protein that is required for transport of phosphate into the cytoplasm. Therefore, uptake, hydrolysis, and periplasmic binding of a phosphate source can take place in the stalk, but high-affinity import must take place in the cell body. Furthermore, by using analytical modeling, we illustrate the biophysical advantage of the stalk as a morphological adaptation to the diffusion-limited, oligotrophic environments where C. crescentus thrives. This advantage is due to the fact that a stalk is long and thin, a favorable shape for maximizing contact with diffusing nutrients while minimizing increases in both surface area and cell volume.

Caulobacter | diffusion | morphology | protein localization | stalk

Bacteria exhibit an amazing diversity of shapes and sizes. Bacterial cells can be round, cylindrical, curved, or coiled. Some are shaped like a flat square, others like a star; some are branched, and some have projections of the cell surface. Exactly how bacterial shapes are generated is not known, and the purposes of a specific cell shape are not always clear. Yet, in most cases, specific shapes are precisely reproduced at every generation. Cell shape changes can also occur during the life cycle of many bacterial species, such as the transformation of Sinorhizobium from rods to branched cells (Y forms) after colonization of legume root nodules. Although these morphological transformations are thought to play important roles in the life cycles of bacteria, the advantages of cell shape changes remain essentially speculative (1).

Here, we investigate the function of prosthecae, cell envelope extensions that are present in a morphologically diverse group of Gram-negative bacteria (2). Unlike flagella or pili, prosthecae are true extensions of the cell proper, possessing both peptidoglycan and cell membranes. In the aquatic bacterium Caulobacter crescentus, prosthecae are referred to as stalks. The function of Caulobacter stalks is not known, but one common hypothesis is that they facilitate the acquisition of nutrients from the environment (3, 4). This hypothesis is consistent with the observation that stalks dramatically elongate under phosphate starvation conditions, with the capacity to reach lengths of >30 μm (5, 6), a length that is approximately equivalent to that of 15 cell bodies. We show that the stalk of C. crescentus (Fig. 1A) can transport a nutrient molecule from the extracellular space into its periplasm and that the stalk constitutes a biophysically efficient morphological adaptation to environments where nutrient uptake is limited by diffusion.

Results

Stalks Can Take Up and Hydrolyze Fluorescein Diphosphate (FDP). To determine whether stalks are capable of nutrient uptake, we incubated cells with FDP, a fluorogenic substrate for the periplasmic enzyme alkaline phosphatase. Fluorescein is liberated when the phosphate-ester bonds of FDP are cleaved; thus, the uptake and hydrolysis of this substrate can be monitored by using epifluorescent microscopy. Two to 5 min after addition of FDP, the perimeter of the cell body and its attached stalk began emitting fluorescence simultaneously (Fig. 2B), suggesting that FDP uptake occurs at a similar rate in the stalk and the cell body. The peripheral fluorescence emission in cell bodies was consistent with the reaction occurring in the periplasm. Similarly, purified stalks began emitting fluorescence within 2–5 min (Fig. 2D), indicating that the stalk alone is capable of FDP uptake and hydrolysis. Stalk fluorescence increased simultaneously along the length of the stalk, indicating that FDP uptake occurred throughout the stalk. Stalks and cells incubated with purified fluorescein never fluoresced (data not shown), indicating that the emission seen in the stalks upon exposure to FDP was not due to a secreted alkaline phosphatase or the uptake and/or nonspecific association of unconjugated fluorescein with the cellular surface. The ability of the stalk to take up FDP but not fluorescein suggests that the stalk possesses receptors or porins with specificity for organic phosphate.

The Stalk Is Compartmentalized from the Cell Body. The organophosphate compound FDP is imported into the stalk periplasm and cleaved by alkaline phosphatase to release inorganic phosphate. What is the fate of this periplasmic phosphate molecule? In cells, periplasmic phosphate is transported across the inner membrane (IM) by the high-affinity phosphate ATP-binding cassette (ABC) transporter (PstSCAB) (6), which is composed of a high-affinity periplasmic phosphate-binding protein, PstS; two IM channel proteins, PstA and PstC; and a cytoplasmic ATPase, PstB. In one scenario, the phosphate released in the stalk periplasm is bound by PstS, and the phosphate–PstS complex diffuses through the stalk periplasm to the cell body periplasm to be taken up through the IM by PstCAB (Fig. 1B). Alternatively, phosphate could be transported across the stalk IM by PstSCAB and then diffuse along the core of the stalk to the cytoplasm of the cell body to be metabolized (Fig. 1B). The first scenario requires that only PstS is present in the stalk periplasm, whereas the second scenario requires that the entire PstSCAB transporter and ATP be present in the stalk.

To determine whether PstS is present in the stalk periplasm and to examine the relative abundance of periplasmic nutrient-binding proteins in the stalk relative to the cell body, M2 epitope fusions were made to PstS (CC1515); a putative phosphonate-binding protein, PhnD (CC0362); and a putative putrescine-binding protein, PotF (CC3137). Western blot analysis showed...
that purified stalks contain significant levels of all three periplasmic binding proteins (Fig. 3). The relative level of the periplasmic nutrient-binding proteins was higher in the stalks than in cell bodies, as would be expected, because stalk fractions contain very little cytoplasmic protein (7). The abundant cytoplasmic protein FtsZ was undetectable in the stalk fractions, indicating that there was no significant contamination of the stalk fractions by cell bodies (Fig. 3, lower blot).

We next asked whether the stalk contained other components of the phosphate ABC transporter by using a PstA–GFP fusion. Fluorescence was easily detected in the cell body (Fig. 4 E and F) but not in stalks. Western blot analysis confirmed that PstA–GFP is found in cell membranes and intact cells but is virtually undetectable in stalk fractions (Fig. 6, which is published as supporting information on the PNAS web site). Because PstS is present in the stalk and PstA is absent, phosphate taken up in the stalk periplasm, either as a free molecule or bound to PstS, must diffuse from the stalk periplasm to the cell body periplasm to be transported into the cytoplasm by the phosphate ABC transporter.

The absence of PstA–GFP from the stalk led us to ask whether other IM proteins were present or absent from the stalk. Immunofluorescence localization of an M2 epitope fusion to the IM protein ExbB, a protein that is required for TonB-dependent transport across the outer membrane (OM) of Gram-negative bacteria, showed that ExbB–M2 was associated with the cell body but was not detectable in the stalk (Fig. 4 B and C) even after long exposures. The absence of two IM proteins, PstA and ExbB, from the stalk is consistent with our previous 2D gel analysis of the stalk proteome, which had identified many periplasmic and OM proteins but essentially no IM proteins (7). Because the IM proteins involved in nutrient uptake are hydrophobic and therefore difficult to identify by using 2D gel electrophoresis, we used 2D liquid chromatography tandem MS (2D LC-MS) to obtain a more complete proteomic profile of purified stalks. The proteomic 2D LC-MS profiling suggested that, in the stalk, OM and periplasmic proteins are more abundant than IM and cytoplasmic proteins contain significant levels of all three periplasmic binding proteins (Fig. 3). The relative level of the periplasmic nutrient-binding proteins was higher in the stalks than in cell bodies, as would be expected, because stalk fractions contain very little cytoplasmic protein (7). The abundant cytoplasmic protein FtsZ was undetectable in the stalk fractions, indicating that there was no significant contamination of the stalk fractions by cell bodies (Fig. 3, lower blot).

We next asked whether the stalk contained other components of the phosphate ABC transporter by using a PstA–GFP fusion. Fluorescence was easily detected in the cell body (Fig. 4 E and F) but not in stalks. Western blot analysis confirmed that PstA–GFP is found in cell membranes and intact cells but is virtually undetectable in stalk fractions (Fig. 6, which is published as supporting information on the PNAS web site). Because PstS is present in the stalk and PstA is absent, phosphate taken up in the stalk periplasm, either as a free molecule or bound to...
mic proteins (Table 1, which is published as supporting information on the PNAS web site). These data indicate that the stalk organelle is at least partially compartmentalized from the cell body with respect to protein composition.

**Stalks Confer a Biophysical Advantage to C. crescentus When Nutrient Uptake Is Limited by Diffusion.** *C. crescentus* stalks may have evolved to enhance nutrient uptake to the cell. Why would growing a stalk be a more advantageous strategy for enhancing nutrient uptake than simply adding more nutrient receptors to the cell body? In the environments where they are found, such as lakes, ponds, or water-saturated soil, *C. crescentus* cells often attach to surfaces by means of a holdfast found at the distal tip of the stalk (Fig. 1A) and typically are not subject to fluid flow. In such diffusion-limited environments, nutrient molecules depend on diffusion for contact with their receptors. Microscopically, the random-walk trajectory of a diffusing molecule explores a given region in space well before wandering away (8). As a result, adding a second receptor to the cell surface will double the rate of uptake only if it is well separated from the first receptor with respect to its size, because a diffusing molecule in the vicinity of the second receptor might instead be absorbed by the first one if they are placed “too close” to each other. This phenomenon is illustrated in Fig. 7, which is published as supporting information on the PNAS web site.

A mathematical analysis (see Supporting Text, which is published as supporting information on the PNAS web site) of diffusive uptake of nutrients to the *C. crescentus* cell body and stalk following Berg and Purcell (9) demonstrates the saturation of uptake capability with increasing numbers of receptors. Considering a range consistent with published abundances of some bacterial OM nutrient receptors (10, 11), we find that doubling the number of receptors on the cell body from 10,000 to 20,000 leads to an increase of only 5% (from 83% to 88%) in the rate of nutrient absorption relative to the maximum rate that is obtained when the entire cell surface is absorptive (Fig. 5A). However, if the additional 10,000 receptors are placed on a stalk instead of the cell body, for stalks of length 1, 5, and 10 μm, the overall rate of uptake is increased by 50%, 140%, and 210%, respectively (Fig. 5B). Thus, incorporating additional receptors on a stalk is a dramatically more efficient strategy for increasing nutrient uptake capacity than incorporating new receptors on a cell body surface among those receptors already synthesized.

In mitigating the saturation to the maximum rate of uptake, is elaborating a stalk preferable to elongating the cell itself? Underlying the results given above and as is easily shown (see ref. 9 and Supporting Text), the rate of nutrient uptake in diffusion-limited environments is proportional to the effective linear dimension of a structure rather than to its surface area. This dependence is in contrast to systems with fluid flow or mixing, such as in the highly evaginated surface of the intestinal epithelium, where uptake is proportional to surface area. For example, a 5.45-μm-long stalk with a diameter of 0.1 μm has the same surface area as a cell body of typical dimensions used in our
The compartmentalization between the stalk and the cell body suggests that nutrient uptake by the stalk is passive, because this type of transport does not require IM or cytoplasmic proteins to provide the energy required. Given that *C. crescentus* lives in oligotrophic environments where the concentration of extracellular nutrients is very low, our results beg the following question: Is passive transport sufficient for efficient nutrient uptake by the stalk in oligotrophic environments? For passive diffusion to be sufficient, the level of periplasmic nutrients must be maintained at lower levels than those found in the environment, and at least two mechanisms would ensure this scenario. First, the periplasmic binding proteins will bind free nutrients with high affinity in the periplasm and thus remove them from the effective concentration. Second, the cell body IM, equipped with active ABC transporters, will act as a nutrient sink, generating a concentration gradient along the length of the stalk. Recent FLIP (fluorescence loss in photobleaching) experiments on the later stages of cell division in *C. crescentus* suggest that the periplasmic spaces of the stalk and cell body are contiguous (see ref. 18 and Fig. 5 A and C). Therefore, it is likely that nutrient molecules absorbed by the stalk OM and bound to periplasmic nutrient-binding proteins diffuse from the stalk periplasmic space into the cell body periplasmic space. Once steady state is achieved in the periplasmic space, the presence of the stalk effectively increases the concentration of nutrient molecules in the cell body periplasm, leading to a greater absorption rate across the cell body IM that is equal to the absorption rate by the stalk OM. The time scale for reaching steady state in the periplasm is estimated to be \( \approx 17 \) and 42 min for stalks with lengths of 1 and 5 \( \mu \)m, respectively (see Supporting Text). This time scale is short compared with the duration of the *C. crescentus* cell cycle, which is on the order of hours under nutrient-deprived conditions. Hence, the stalk would quickly enhance the rate of nutrient uptake into the cytoplasm by an amount that is equal to the stalk’s rate of uptake from the environment.

One question that is particularly intriguing is how nutrient molecules might be transported across the *C. crescentus* OM, particularly in stalks. Interestingly, the *C. crescentus* genome lacks sequence homologs to any of the major general porins (such as OmpF and OmpC) that are found in many other bacteria. There are 65 putative TonB-dependent receptors in *C. crescentus*, and TonB-dependent receptors make up a predominance of the proteins identified in stalk fractions, as shown in this study and in ref. 7. However, because ExbB, an IM protein that is required for TonB-dependent transport, is absent from the stalks, they are unlikely to be capable of TonB-dependent transport, at least as it is currently understood. In the future, it will be of interest to determine what proteins are required for the uptake of nutrients by the stalk as well as whether the mechanism is passive or requires energy.

In summary, the increase in stalk length upon phosphate starvation (6), the ability of stalks to take up and hydrolyze a phosphate ester compound, the presence of high-affinity periplasmic nutrient-binding proteins in the stalk, the protein compartmentalization between the stalk and cell body, and the advantage of placing additional nutrient receptors on a linear appendage while minimizing increases in surface area and volume all support the hypothesis that stalk formation is a morphological adaptation that enhances nutrient uptake in oligotrophic environments.

Materials and Methods

**Bacterial Strains, Plasmids, Oligonucleotides, and Growth Conditions.** The strains and plasmids used in this study are provided in Table 2, which is published as supporting information on the PNAS web site. All cultures were grown at 30°C in either PYE (19) or HIGG (20) medium containing 120 \( \mu \)M phosphate. When appropriate, liquid cultures were supplemented with 5 \( \mu \)g/ml...
kanamycin. The cultures from which stalks were harvested were grown to stationary phase. Oligonucleotides used in this study are listed in Table 3, which is published as supporting information on the PNAS web site.

**Sample Preparation.** All steps were carried out on ice or at 4°C. To collect stalks by shearing, cultures were grown in HIGG medium containing 120 μM phosphate, chilled on ice for 10–20 min, and blended for 3 min in an ice-cold Waring blender as described in refs. 21 and 22. This stalk removal method does not lead to significant leakage of periplasmic or cytoplasmic proteins from samples, and cell bodies have no detectable loss in viability (22). For stalk purification, the cells were pelleted by centrifugation at 10,410 × g for 15 min. The supernatants, excluding the loose pellet, were again centrifuged at 10,410 × g for 15 min to remove more cell bodies. The supernatants, excluding the loose pellets, were divided into Oakridge tubes and centrifuged at 38,720 × g for 30 min to pellet the stalks. To further enrich for stalks free of cell bodies, only the fluffy outer portion of the pellet in each tube was resuspended. The first pellet was resuspended in 0.5 ml of cell bodies, only the fluffy outer portion of the pellet in each tube was resuspended. The second pellet was resuspended in 10 ml of ddH2O. Microscopic examination determined the extent of cell body contamination, which was generally very rare and ranged from one to five cell bodies per 2,000 stalks (data not shown). To prepare the cell bodies relatively free of unattached stalks, one of the pellets from the first centrifugation was resuspended in ~250 ml of high basal salts without phosphate buffer (20), and this suspension was centrifuged at 10,410 × g for 10 min. The supernatant was poured off, and the pellet was washed once with ddH2O and resuspended in 10 ml of ddH2O. Microscopic examination confirmed that the bodies had only one to two free stalks per 200 cell bodies (data not shown).

**Microscopy.** Phase-contrast and epifluorescence microscopy were performed on a Nikon Eclipse E800 light microscope equipped with a ×100 Plan Apo objective and a cooled charge-coupled device camera (model 1317, Princeton Instruments, Trenton, NJ). Images were captured and analyzed by using METAMORPH 4.6 imaging software (Universal Imaging, Downingtown, PA). 2D deconvolution was performed by using the METAMORPH software “nearest neighbors” function with the following settings: filter size, 9; scaling factor, 0.97; result scale, 5; suppress noise, on; autoscale result, on.

**In Vivo Experiments with FDP.** To examine the ability of cells and stalks to hydrolyze the chromogenic substrate FDP (Molecular Probes), FDP was added to a final concentration of 100 μM, and samples were mounted on a slide and observed under ×1,000 magnification by using phase and fluorescence with an FITC filter. Two to 5 min passed from the addition of substrate to the time of image capture. The experiment with fluorescein confirmed that the bodies had only one to two free stalks per 200 cell bodies (data not shown).

**Western Blot Analysis.** The M2 epitope-tagged proteins were detected by using a 1:750 or 1:1,000 dilution of anti-M2 polyclonal antibody conjugated to horseradish peroxidase (Sigma). The GFP-tagged proteins were detected by using a 1:5,000 dilution of monoclonal anti-GFP antibody (Clontech) and a 1:20,000 dilution of horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Bio-Rad) preincubated for 15 min with NA1000 acetic acid powders (23). Western blot images were captured with a Kodak Image Station 440 CF.

**Immunofluorescence Microscopy.** Immunofluorescence microscopy was performed by fixing cells in 2.5% (vol/vol) formaldehyde and 30 mM PBS (pH 7.5). All centrifugations were carried out at 7,000 rpm in a Sorvall Biofuge Pico table-top microcentrifuge for 7 min at room temperature. All washes were in a 1.0-ml volume unless indicated otherwise. The cells were washed twice with PBS and incubated with 100 μl of 0.1% (vol/vol) Triton X-100 in PBS for 45 min at room temperature. The cells were washed three times with PBS and resuspended in 100 μl of 5.0 mM EDTA in PBS for 20 min at room temperature. Lysozyme (Sigma) was added to a final concentration of 1.0–2.0 ng/μl, and the cells were monitored by microscopy until they became slightly transparent. The cells were washed three times with PBS to remove the lysozyme and incubated with 100 μl of blocking reagent [0.5% (wt/vol) Roche Molecular Biochemicals Blocking Reagent in PBS] at 37°C for 30 min. Anti-M2 polyclonal antibody (Sigma) was added to a final concentration of 1:250, and the cells were returned to 37°C for 1 h. The cells were washed three times with PBS containing 0.05% (vol/vol) Tween 20 and then resuspended in 100 μl of blocking reagent containing a 1:100 dilution of FITC-conjugated, affinity-purified goat anti-rabbit secondary antibody (Jackson ImmunoResearch) and incubated at 37°C for 30 min. Cells were then washed three times with PBS containing 0.05% (vol/vol) Tween 20 and resuspended in 5.0 μl of Slowfade Reagent A (Molecular Probes). Samples were kept on ice in the dark before microscopy.

**2D Liquid Chromatography Tandem MS Analysis.** Stalks were purified as described above, except that they were resuspended in 0.25–0.5 ml of TE (10 mM Tris/1 mM EDTA, pH 8.0) or doubly distilled H2O. Also, stalk samples were incubated for 30 min to 1 h on ice with 16 μg/ml lysozyme (Sigma). The concentration of protein in the purified stalks was determined by the method of Bradford (24). One hundred fifty micrograms of stalk protein in 50 mM ammonium bicarbonate and 0.5–1.0% (wt/vol) RapiGest (Waters) was boiled for 5 min. The sample was allowed to cool to room temperature, dithiothreitol was added to a final concentration of 5 mM, and the sample was placed at 60°C for 30 min. The sample was cooled to room temperature, iodoacetamide was added to a final concentration of 15 mM, and the sample was placed in the dark for 30 min at room temperature. Trypsin (Sigma) was added at a ratio of 1 μg of trypsin::50 μg of stalk protein, and the sample was incubated at 37°C for a minimum of 16 h. To remove the RapiGest, HCl was added to a final concentration of 40 mM, and the sample was placed at 37°C for 30 min. This step precipitated the detergent. To remove the detergent and any debris present, the samples were centrifuged for 40 min at 30,000 rpm by using a TLA 100 rotor in a Beckman Optima TLX ultracentrifuge at 4°C.

The strong cation exchange column used in all experiments was 10 cm × 254 μm i.d. and was packed with 5-μm silica beads with a benzene sulfonic acid functional group (Phenomenex, Torrance, CA). In all cases, a 1-h linear gradient from 0 to 200 mM sodium perchlorate in 0.1% formic acid and 50% acetonitrile was used. The flow rate was 5 μl/min in each experiment. Approximately 20 μg of tryptic digest, as determined from a Bradford assay, was injected onto the SCX column in each experiment. Column effluent was collected manually in 2-min fractions for the duration of the gradient. Each fraction was subsequently diluted to reduce the acetonitrile concentration by adding 40 μl of 0.1% trifluoroacetic acid before reverse-phase analysis.

The reverse-phase column used in all experiments was packed with 5-μm C18 derivatized silica beads and was 10 cm × 254 μm. The diluted fractions collected in the strong cation exchange dimension were injected onto the reverse-phase column without further purification. The gradient in all reverse-phase experiments increased linearly from 5% to 40% of 0.1% formic acid in
acetonitrile. The six ion-exchange fractions with the highest mass spectrometric ion yield were separated with a 6-h gradient. The six fractions with the next highest ion yield were separated with a 2-h gradient. All others were fractionated with a 1-h gradient. The reverse-phase column effluent was directly analyzed by using a LCQ Deca XP ion trap mass spectrometer (Thermo Electron, San Jose, CA) with an electrospray source.

The mass spectrometer was operated in data-dependent mode with three MS/MS spectra recorded for every MS spectrum. In this mode, the three most intense peaks in the MS spectrum are chosen for fragmentation. These parent masses are then placed on an exclusion list for 3 min. Mass spectra were collected for the duration for fragmentation. These parent masses are then placed on an exclusion list for 3 min. Mass spectra were collected for the duration of each reverse-phase experiment by using the XCALIBUR software package operated in centroid mode.

**SEQUEST Analysis.** The results of all experiments were analyzed by using the SEQUEST search algorithm. The data were searched against a database of *C. crescentus* proteins obtained from SwissProt with the sequence for egg white lysozyme manually inserted, which yielded a total of 3,750 proteins in the database. Because the sample was reduced and alkylated, all cysteine residues were assumed to be acetamidated. All analyses assumed tryptic enzyme activity with a maximum of two missed cleavages. To be considered acceptable peptide identification, the crosscorrelation values had to be at least 2.0, 2.5, or 3.5 for charge states of +1, +2, and +3, respectively. These criteria have been previously shown to be quite stringent (25). All mass spectra meeting these minimum criteria were also manually validated. Peptides that contained overlapping sequence coverage due to missed cleavages were counted as separate peptide identifications. At least two peptides from a protein had to be detected in order for that protein to be considered identified.

Y.V.B. dedicates this article to the loving memory of his father, Omer Brun (1926–2006), who playfully taught a young boy to seek scientific explanations to natural phenomena. We thank Howard Berg for helpful discussions; members of Y.V.B’s laboratory, Daniel Kearns, Howard Berg, Harley McAdams, Kevin Young, Ned Wingreen, Volkmar Braun, Lucy Shapiro, and Clay Fuqua for critical reading of early versions of the manuscript; and L. Comolli and H. McAdams for communicating results before publication. This work was supported by National Institutes of Health Grants GM51986 (to Y.V.B.) and GM61336 (to J.P.R. and Y.V.B.) and by National Institutes of Health Predoctoral Fellowship GM07757 (to J.K.W.).

Supporting Text

S1 Mathematical Analysis of the *C. crescentus* Stalk as a Diffusion Antenna for Nutrient Uptake

The biochemical and proteomic analysis of the *C. crescentus* stalk presented in the main text of this report suggests a direct role in enhancing nutrient uptake. Although increasing receptor surface area is the preferred means for increasing uptake capability in the presence of fluid flow, this is not the case when nutrient uptake is predominantly due to diffusion. In this section, we demonstrate mathematically how growing a stalk represents a beneficial strategy for *C. crescentus* in a diffusion-limited environment by facilitating an optimal arrangement of nutrient receptors. Some of the discussion in the main text of the report is repeated here, so that the present material can be read as a stand-alone document.

S1.1 Summary of Results

In a diffusion-limited environment, simply increasing the number of receptors on the *C. crescentus* cell body may not be the most effective strategy for enhancing nutrient uptake. This can be illustrated by considering the path of a diffusing particle. Microscopically, the random walk trajectory of a diffusing particle explores a given region in space well before wandering away (1). This qualitative picture has important consequences for the rate of uptake (number of particles absorbed per unit time), when particles are dependent on diffusion for contact with their absorbers (i.e., nutrient receptors). For example, given a disk-shaped receptor, adding a second absorber to the cell surface will double the rate of uptake only if it is well separated from the first one, compared to their size. This is because a diffusing particle in the vicinity of the second absorber might instead be absorbed by the first one if the absorbers are placed “too close” to each other, as illustrated in Fig. 7.

The rate of diffusive uptake by *N* discrete disk-like absorbers on an otherwise nonabsorbing spherical surface was first addressed by Berg and Purcell (2) in their study of the physical limits to the measurement of the concentration of diffusing signaling molecules by
surface receptors in single-celled organisms (for example, in bacterial chemotaxis). They showed that for absorbers of radius, $s$, distributed uniformly on the surface of a sphere of radius, $R$, where $R \gg s$, the rate of uptake is initially proportional to $N$ (for $Ns \ll R$) but eventually saturates (for $Ns \sim R$) to the maximum rate that results when the entire surface is covered by absorbers. The individual absorbers are assumed to be perfect in that upon contact with a diffusing molecule, the probability of absorption is equal to one. Treatment of nonperfect absorbers is equivalent to reducing their size, $s$. In the context of *C. crescentus* stalk formation, we extend this work to show that for the cell to increase its rate of nutrient uptake by diffusion, it is advantageous to arrange additional receptors onto an auxiliary structure such as a stalk. Details of this calculation are given in Section S1.2.

The stalk and cell body can be approximated as prolate spheroidal bodies of dimensions $(b, b, \ell)$, with $\ell > b$, as indicated in Fig. 7. The maximum rate of uptake $I_{\text{spheroid}}^{\text{max}}$ is obtained from the solution to the steady state diffusion equation for the substrate concentration in three dimensions in prolate spheroidal coordinates, subject to the boundary conditions of zero concentration, $c(r, t) = 0$, at the absorbing surface, and constant concentration $c_0$ infinitely far from the surface:

$$I_{\text{spheroid}}^{\text{max}}(\ell, b) = \frac{4\pi Dc_0\ell}{\tanh^{-1} \left( \frac{1 - b^2/\ell^2}{\ell^2} \right)} = 4\pi Dc_0\ell_{\text{effective}}.$$  

We note that the maximum rate of uptake to the surface is proportional to an effective linear dimension of the body, $\ell_{\text{effective}} \equiv (e/\tanh^{-1} e) \ell$, where $e = \sqrt{1 - b^2/\ell^2}$ is the eccentricity of the spheroid. This is also true for other shapes when uptake is diffusion limited.* In contrast, when the dominant transport of particles to the absorber is not by diffusion, but rather by fluid advection or mixing, then the rate of uptake is no longer proportional to the length of the absorber but rather to its surface area.†

*From dimensional analysis, the rate of diffusive uptake must be proportional to both the diffusion constant and the concentration of substrate molecules, $I \propto Dc_0$, where $I$ has dimensions of number of particles per unit time, $D$ has dimensions of length$^2$ per unit time and $c_0$ has dimensions of number of particles per length$^3$. Hence, $I$ must also depend on the effective length of the absorber, $\ell_{\text{effective}}$, giving $I \sim Dc_0\ell_{\text{effective}}$.

†Again, from dimensional analysis, we must have $I \sim u c_0 A$, where $u$ is the fluid velocity in units of...
We address how this maximum rate of uptake is modified with $N$ discrete disk-like absorbers of radius $s$, by calculating the probability $P_{\text{esc}}$ that a diffusing particle that collides with the surface will eventually escape to infinity and not be captured by any of the absorbers. Our analysis extends Berg and Purcell’s calculation for the spherical case (2) to the prolate spheroidal geometries that characterize the $C. \text{ crescentus}$ cell body and stalk (see Section S1.2). The modified rate of uptake is given by $I_{\text{spheroid}}(N; \ell, b, s) = I_{\text{max spheroid}}(\ell, b)(1-P_{\text{esc}})$. In the limit of a “cigar-shaped” absorbing surface such as the stalk, where $b \ll \ell$, we have

$$1 - P_{\text{esc}} \approx \left[1 + \frac{4\ell}{Ns} \ln \left(\frac{2\ell}{b} - \frac{4s}{\pi b}\right)\right]^{-1},$$

and in the limit of an absorbing surface such as the cell body, where $b \approx \ell$, we have

$$1 - P_{\text{esc}} \approx \left[1 + \frac{4b^2}{N\ell s} \frac{1}{1 - \frac{2bs}{\ell(b+\ell)}}\right]^{-1}.$$

We note that the saturation of the modified rate of uptake to the maximum rate with increasing $N$ depends on the ratio of the linear size of the single discrete absorber to an effective linear size of the spheroid.

In the main text (see Fig. 5A), we have plotted the rate of uptake by the cell body with $N$ discrete absorbers, $I_{\text{cell}}(N) = I_{\text{spheroid}}(N; \ell_{\text{cell}}, b_{\text{cell}}, s)$ as a function of $N$ in units of the maximum rate of uptake by the cell body, $I_{\text{cell}} = I_{\text{max spheroid}}(\ell_{\text{cell}}, b_{\text{cell}})$, using typical dimensions for the $C. \text{ crescentus}$ cell body, where $(\ell_{\text{cell}}, b_{\text{cell}}) = (0.5, 0.25) \ \mu\text{m}$ and typical sizes of transport porins, $s = 1 \ \text{nm}$ (3, 4). Increasing the number of absorbers on the cell body by 100% from $N = 10,000$ to $N = 20,000$ leads to a 5% increase, from 83% to only 88% in the rate of nutrient absorption relative to the maximum rate.

We also plotted (see Fig. 5B in the main text) the rate of uptake by the stalk with $N$ discrete absorbers, $I_{\text{stalk}}(N) = I_{\text{spheroid}}(N; \ell_{\text{stalk}}, b_{\text{stalk}}, s)$, as a function of $N$ for typical stalk dimensions, in units of the maximum rate of uptake by the cell body. If the $N = 10,000$ new receptors are placed on a stalk instead of the cell body, for stalks of length $L = 2\ell_{\text{stalk}}$ length per unit time, $c_0$ is the concentration in units of number of particles per length$^3$, and $A$ is the area of the absorber in units of length$^2$. 

S3
equal to 1, 5, and 10 µm, the rate of uptake is increased by approximately 50%, 125%, and 200%, respectively, of the maximum rate of uptake by the cell body.

For comparison, in Fig. 8A, we plot the rate of uptake by an elongated cell with \( N \) discrete absorbers, \( I_{\text{elongated cell}}(N) = I_{\text{spheroid}}(N; f_{\ell \text{cell}}, b_{\text{cell}}, s) \), as a function of \( N \) in units of the maximum uptake by the cell body for different cell lengths, where \( L_{\text{cell}} = 2f_{\ell \text{cell}} \), and \( f \) is a scale factor. In Fig. 8B, we plot the rate of uptake for a stalked cell, \( I_{\text{stalked cell}}(N) \), with \( N \) discrete absorbers uniformly distributed on the cell body and stalk surfaces. The length of the cell body is held fixed (with \( L_{\text{cell}} = 1 \) µm), while the stalk length is varied to achieve the same total length as the hypothetical elongated cell in Fig. 8A.

We note that the corresponding curves in Fig. 8A and Fig. 8B indicate that the absorption rate for stalked cells is comparable to (though slightly less than) that for elongated cells of the same total length. This is true despite the fact that the surface areas of elongated cells with total lengths of 3, 5, and 10 µm are approximately 2, 2.5, and 3.5 times that of stalked cells of the same length. Strikingly, as shown in Fig. 9, the maximum rates of uptake per unit volume and uptake per unit surface area are significantly greater for stalked cells than for elongated cells. Therefore, growing a stalk would be an efficient strategy for a cell to increase its rate of nutrient uptake while at the same time minimizing the cost of increasing both surface area and volume.

**S1.2 Modified Rate of Uptake of an Ellipsoidal Object with \( N \) Discrete Absorption Sites**

For an ellipsoidal absorber, assumed for simplicity to be a prolate spheroid with dimensions \((b, b, \ell)\), the total rate of uptake is obtained by solving Laplace’s equation in three dimensions in prolate spheroidal coordinates subject to boundary conditions given by \( c(r, t) = 0 \) at the surface of the absorber and \( c(r, t) = c_0 \) infinitely far from the absorber

\[
I_{\text{spheroid}} = \frac{4\pi D_{c_0} \ell \sqrt{1 - b^2/\ell^2}}{\tanh^{-1} \left( \sqrt{1 - b^2/\ell^2} \right)}. \tag{4}
\]

With \( N \) discrete disk-like absorbers of radius \( s \), to determine how this maximum rate of
uptake is modified we first find the probability that a diffusing particle at a given location in space reaches the surface of the spheroid: this is given by the solution to Laplace’s equation in prolate spheroidal coordinates subject to boundary conditions \( P = 0 \) infinitely far from the surface, and \( P = 1 \) at the surface of the spheroid

\[
P(\xi) = \frac{1}{\tanh^{-1} \sqrt{1-b^2/\ell^2}} \tan^{-1} \sqrt{\frac{1-b^2/\ell^2}{1+\xi/\ell^2}},
\]

where prolate spheroidal coordinates are \( \{\xi, \zeta, \phi\} \)

\[
\begin{align*}
\xi &= \frac{1}{2} \left[ -(\ell^2 + b^2) + \rho^2 + z^2 + \sqrt{-4\ell^2(b^2 - \rho^2) + 4b^2z^2 + (\ell^2 + b^2 - \rho^2 - z^2)^2} \right], \\
\zeta &= \frac{1}{2} \left[ -(\ell^2 + b^2) + \rho^2 + z^2 - \sqrt{-4\ell^2(b^2 - \rho^2) + 4b^2z^2 + (\ell^2 + b^2 - \rho^2 - z^2)^2} \right], \\
\phi &= \tan^{-1} \frac{y}{x},
\end{align*}
\]

with \( \xi \geq -\ell^2, -\ell^2 \leq \zeta \leq -b^2, 0 \leq \phi \leq 2\pi, \) and \( \rho^2 = x^2 + y^2 \). The surface of the spheroid in Cartesian coordinates

\[
\frac{x^2}{b^2} + \frac{y^2}{b^2} + \frac{z^2}{\ell^2} = 1,
\]

is given in prolate spheroidal coordinates by \( \xi = 0 \). If the entire surface is absorbing, then the probability that a particle at \( \xi(\rho, z) \) will be absorbed is given by Eq. 5. What if there are \( N \) disk shaped absorbers of radius \( s \), while the rest of the surface is non-absorbing? Berg and Purcell (2) addressed this question for the spherical case in their analysis of the physical limits to biochemical signaling in the context of bacterial chemotaxis. Their assumption, which we likewise follow here, is that a particle in the vicinity of the spheroid is destined to make many encounters with the surface before escaping to infinity, if at all. Assuming the absorbers are uniformly distributed on the surface of the spheroid of surface area \( A \), the probability that a given encounter is not with an absorber is \( (1 - N\pi s^2 / A)P \equiv \beta P \), where the surface area of a prolate spheroid is

\[
A = 2\pi b^2 + \frac{2\pi \ell b}{\sqrt{1 - b^2/\ell^2}} \sin^{-1} \sqrt{1 - b^2/\ell^2},
\]

with

\[
A \approx \pi^2 \ell b,
\]

S5
\[ \approx \frac{2\pi b(b + \ell)}{b \sim \ell} . \quad (9) \]

We would like to find the probability that the particle does not encounter an absorber after \( n = 0, 1, \ldots \infty \) number of independent hits. For the purpose of an approximate calculation, we define independent hits with the surface as those separated by a distance on the surface approximately equal to \( s \). Following Berg and Purcell, we assert that consecutive hits are independent provided that they are separated by an excursion above the surface by a distance also approximately equal to \( s \). Hence, the probability that a particle in the vicinity of the surface - defined to be a perpendicular distance approximately equal to \( s \) above the surface - will escape to infinity is given by

\[
P_{esc} = \sum_{n=0}^{\infty} \left[ (\beta P_s)^n \cdot (1 - P_s) \right] = \frac{1 - P_s}{1 - \beta P_s} , \quad (10)
\]

where \( P_s \) is the probability of encountering the surface from a distance \( s \) above it. To determine what this distance corresponds to in prolate spheroidal coordinates, we note that the element of length in the \( \xi \)-direction (perpendicular to the spheroidal surface, \( \xi = \text{constant} \)) is given by \( h_\xi d\xi \), where the scale factor \( h_\xi \) is

\[
h_\xi = \frac{\sqrt{\xi - \zeta}}{2\sqrt{(\xi + \ell^2)(\zeta + b^2)}} . \quad (11)
\]

Hence, for

\[
s \sim h_\xi(\xi = 0)\delta\xi = \frac{\sqrt{-\zeta}}{2\ell b} \delta\xi , \quad (12)
\]

the value of \( \delta\xi \) corresponding to a perpendicular distance \( s \) above the surface varies with position. Therefore, we consider the average value over the surface of spheroid

\[
\langle \delta\xi \rangle = 2\ell b s \left\langle \frac{1}{\sqrt{-\zeta}} \right\rangle , \quad (13)
\]

where

\[
\left\langle \frac{1}{\sqrt{-\zeta}} \right\rangle = 4\pi b/A . \quad (14)
\]
Using Eqs. 8 and 9, we find
\[
\langle \delta \xi \rangle \approx \frac{8bs}{\pi}, 
\]
\[
\approx \frac{4b\ell s}{b + \ell}.
\]
Therefore, we take \( P_s = P(\xi = \langle \delta \xi \rangle) = P(8\pi \ell b^2 s/A) \), which for \( s/b \ll 1 \), becomes
\[
P_s \approx 1 - \frac{4s}{\pi b \ln (2\ell/b)},
\]
\[
\approx 1 - \frac{2bs}{\ell (b + \ell)}.
\]
Finally, with \( N \) discrete absorbers the total rate of uptake by the spheroid is modified by the factor \( (1 - P_{esc}) \)
\[
I_{\text{spheroid}}(N; \ell, b, s)/I_{\text{spheroid}}^{\text{max}}(\ell, b) = \frac{(1 - \beta) P_s}{1 - \beta P_s},
\]
where \( \beta = 1 - N\pi s^2/A \) carries the dependence on \( N \), as defined earlier in this section.

S1.3 Discussion

Diffusion in the Periplasmic Space. Although absorption of nutrient molecules from the environment into the cell body cytoplasm takes place via the two-stage process described in the main text of this report (first, absorption by OM receptors followed by diffusion in the stalk and cell body periplasmic spaces, and then absorption by cell body IM transporters) the role of the stalk is simply to increase the rate of absorption into the cytoplasm where the nutrients will be metabolized. At steady state, the number of nutrient molecules in the periplasm is fixed, and the rate of uptake from the periplasm by the cell body IM is equal to the sum of the rates of uptake by the cell body and stalk OM’s, which we have calculated. The time scale \( \tau \) for reaching steady state depends on the diffusion constant of the nutrient in the periplasm, and is given by \( \tau \sim L_{\text{stalk}}^2/D_{\text{periplasm}} \) for quasi-one dimensional diffusion in the stalk. Diffusion constants of typical proteins in the cytoplasm and in the lipid membrane have been measured in recent experiments, with \( D_{\text{membrane}} = (1.2 \pm 0.2) \times 10^{-2} \, \mu m^2/sec \)
and $D_{\text{cytoplasm}} = (2.5 \pm 0.6) \, \mu m^2/sec$ (7). Earlier measurements on diffusion of fluorescently labeled maltose-binding protein in the *Escherichia coli* periplasm indicate $D_{\text{periplasm}}$ to be comparable to $D_{\text{membrane}}$ (8). Using this estimate for $D_{\text{periplasm}}$, we find that $\tau \sim 17$ and 42 min for $L_{\text{stalk}} = 1$ and 5 $\mu$m, respectively. Should $D_{\text{periplasm}}$ be larger, for example by an order of magnitude, these characteristic diffusion time scales will be reduced by a factor of 10. Even for long stalks, this time scale is short compared to the duration of the *C. crescentus* cell cycle, which is on the order of hours under nutrient deprived conditions. Hence, the stalk quickly enhances the rate of nutrient uptake into the cytoplasm by an amount equal to the stalk’s rate of uptake from the environment.

**Alternate Possibilities for Stalk Function.** Could the stalk play other roles in addition to that of a “diffusive antenna”? Suppose that, in addition to diffusion, the substrate is also advected by fluid flow. At the stationary surface to which the cells are adhered, the bulk fluid velocity decreases to zero within a boundary layer whose thickness depends on the fluid properties. The rate of uptake in the presence of flow is proportional to $uAc_0$, where $u$ is the flow velocity, $A$ is the surface area of the absorber, and $c_0$ is the nutrient concentration. Therefore, the cell body, by virtue of its greater surface area, will have a larger rate of nutrient uptake than the stalk, and a possible additional role of the stalk would then be to lift the cell body out of the boundary layer and into the bulk where the flow velocity is largest. A second possible role for the stalk as a “stem” arises from consideration of the diffusive boundary layer at the surface. As the number of cells attached to a stationary surface becomes appreciable, the concentration of nutrients at the surface decreases from that in the bulk over a thickness proportional to the cell size. Hence, the role of the stalk may additionally be to lift both itself and the cell body into the bulk where the nutrient concentration is less depleted (while the part of the stalk remaining in the cell biomass at the surface could take up nutrients generated by the other cells adhering to the surface). In these ways, prosthecae in the form of “stems” may play additional roles beyond that of
linear appendages that simply project out from anywhere on the cell surface and serve as diffusive antennae.

References


**A**

- Graph showing the relationship between the number of receptors, $N$, and the normalized value, $I_{cell}(N)/I_{cell}^{\text{max}}$, for different cell lengths $L_{cell}$.

- $L_{cell} = 10 \mu m$ (blue line), $L_{cell} = 5 \mu m$ (green line), $L_{cell} = 3 \mu m$ (red line).

- $I_{cell}(N)$ and $I_{cell}^{\text{max}}$ are normalized values.

**B**

- Graph showing the relationship between the number of receptors, $N$, and the normalized value, $I_{stalked\ cell}(N)/I_{cell}^{\text{max}}$, for different stalk lengths $L_{stalk}$.

- $L_{stalk} = 9 \mu m$ (blue line), $L_{stalk} = 4 \mu m$ (green line), $L_{stalk} = 2 \mu m$ (red line).

- $I_{stalked\ cell}(N)$ and $I_{cell}^{\text{max}}$ are normalized values.
A

Max. rate of diffusive uptake per unit area

- Stalked cell
- Elongated cell

Total length, µm

B

Max. rate of diffusive uptake per unit volume

- Stalked cell
- Elongated cell

Total length, µm