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Morphology of *Caulobacter crescentus* and the Mechanical Role of Crescentin

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ABSTRACT Bacterial cells exist in a wide variety of shapes. To understand the mechanism of bacterial shape maintenance, we investigate the morphology of *Caulobacter crescentus*, which is a Gram-negative bacterium that adopts a helical crescent shape. It is known that crescentin, an intermediate filament homolog of *C. crescentus*, is required for maintaining this asymmetrical cell shape. We employ a continuum model to understand the interaction between the bacterial cell wall and the crescentin bundle. The model allows us to examine different scenarios of attaching crescentin to the cell wall and compute the shape of the bacterium. Results show that if the sole influence of crescentin is mechanical, then the crescentin bundle is unrealistically rigid and must be attached to the cell wall directly. The model suggests that alternative roles for crescentin such as how it influences cell wall growth must be considered.

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Bacterial cells exist in a kaleidoscope of shapes, ranging from spheres to rods to more exotic helical filaments. Understanding how cells adopt and maintain these shapes at the molecular level is an important question in cell and developmental biology. A number of bacterial cytoskeletal proteins such as FtsZ and MreB have been found to influence the cell shape. The former is responsible for dividing the cell, and the latter is responsible for maintaining the rodlike appearance of several bacteria (see (1) and references therein). For the Gram-negative *Caulobacter crescentus*, another cytoskeletonlike protein, crescentin, was found to influence the crescentlike shape of the cell. Crescentin is an intermediate filament (IF) homolog of *C. Crescentus*; knockout of this protein changes the helical shape of the cell to a rodlike shape (2). In this letter, we investigate the morphology of *C. crescentus* and the effect of crescentin on the shape of *C. crescentus* using mechanical modeling. Results reveal that the crescentin bundle is unlikely to deform the cell mechanically. A more plausible scenario is that the bundle influences the growth pattern of the cell wall.

Though its x-ray structure has not been revealed, crescentin is classified as a coiled coil, similar to the eukaryotic IF. A bundle of crescentin is therefore mechanically rigid and resists stretching, bending, and twisting. When attached to the flexible cell wall, the situation is similar to a flexible wire attached inside a balloon: the cell will deform according to the mechanical properties of the crescentin bundle. Using known properties of the cell wall, and assuming that the cell wall and the crescentin bundle are static, one can compute the shape of the cell. The shape of the cell will depend on the rigidity and the intrinsic shape of the crescentin bundle, the mechanical properties of the cell wall, and how the bundle is attached to the wall. These factors must be combined in a quantitative model.

Mechanical properties of the Gram-negative bacterial cell wall have been studied (3). The wall can be described by a material with Young's modulus between $Y = 1$–30 MPa. The thickness is $d_t = 6$–7 nm. The cell is also under constant internal turgor pressure of ~0.3 MPa. (Detailed summary of mechanical parameters are given in the Supporting Material.) If the crescentin bundle is intrinsically curved (e.g., helical), then the cell wall will bend to accommodate the shape of bundle. We can use known properties of the coiled coil to estimate the lower-bound size and rigidity of the bundle. We begin by assuming that just as in IFs, the crescentin filament in the cell is a bundle of coiled-coil filaments. We consider a maximally rigid bundle where long continuous coiled coils may be cross-linked by specific cross-linkers, or interact with each other through nonspecific bundling interactions. If the coiled coils interact with each other, then the bending stiffness, a_0, of the crescentin bundle scales as the number of coiled-coil filaments to a power ν (4–6),

$$a_0 = L_p k_B T \times N_c^{\nu}, \tag{1}$$

where $L_p \sim 160$ nm denotes the bending persistence length of the coiled coil (6,7), and ν varies between 1 and 2. For crescentin to mechanically bend the cell significantly, a_0 must be similar to the bending rigidity of the cell wall. Therefore, treating the cell wall as a hollow tube gives

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$$a_0 \approx Y\pi\left[R^4 - (R - d_t)^4\right]/4, \tag{2}$$

where R is the approximate radius of the cell. Combining Eqs. 1 and 2 for $\nu = 2$, i.e., a maximally rigid bundle, gives $N_c \sim 1800$–10,000. Each coiled coil has a cross-sectional area of ~2 nm^2. Therefore, the bundle of crescentin would have a radius of ~35–81 nm. Thus, a maximally rigid bundle of continuous coiled coils may deform the cell mechanically. Intermediate filaments, however, consist of short coiled-coil segments (unit length filaments) that assemble to form long bundles. The stiffness of such a structural arrangement is determined by the strength of bonds between coiled coils, and would be significantly softer than bundles of long continuous coiled coils. If crescentin has a similar makeup, then the bundle must contain significantly more filaments and be >80 nm in radius. Such a large filament bundle has not been seen.

The actual shape of the cell will depend on how the crescentin bundle is attached to the cell wall. In particular, we wish to understand why in starved conditions where some cells can grow longer, *C. crescentus* adopts a helical shape (2,8). The wall of *C. crescentus* can be modeled as a two-dimensional continuum surface in three-dimensional space. To describe cell shape changes, we need two configurations: a reference configuration that is the cell shape without any forces and internal pressure, and a current configuration that is the deformed shape after the application of other forces and turgor pressure. We describe each configuration with a center curve and a radius at each point of the curve (Fig. 1). We denote the material points on the reference cell surface as $\boldsymbol{p}$. For each material point $\boldsymbol{p}$, we have a local frame formed by the tangent vectors on the surface, $\boldsymbol{p}_1$ and $\boldsymbol{p}_2$, and the surface normal $\boldsymbol{n} \propto \boldsymbol{p}_1 \times \boldsymbol{p}_2$. Mechanical deformations are described by the current shape, $\boldsymbol{p}'$, and the current normal vector, $\boldsymbol{n}'$ is defined in the same way as above (Fig. 1). Precise definitions of these quantities are given in the Supporting Material.

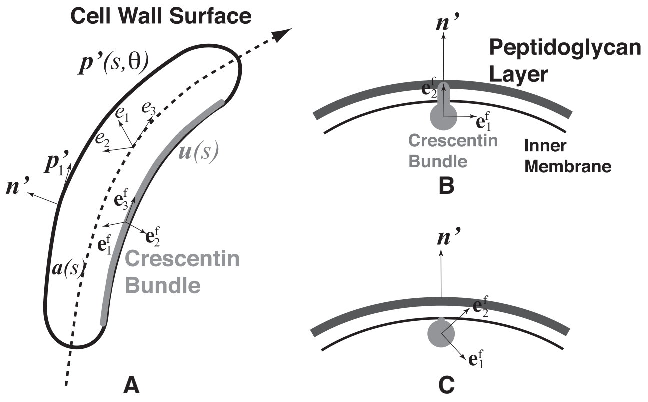

FIGURE 1 (*A*) The shape of the cell is described by the cell surface p' and the crescentin bundle u. A full geometrical description is given in the Supporting Material. (*B*) The interaction between the crescentin bundle and the cell body is determined by how the bundle is attached to the cell wall. If the bundle is attached rigidly to the wall, then the twist of the bundle (described by $e^f{}_1$, $e^f{}_2$) is coupled to the surface normal, n′, of the wall. (*C*) If the bundle is anchored to the soft inner membrane, the cell wall is unaffected by twist in the bundle.

The crescentin bundle is seen at one side of the cell (Fig. 1), although the exact manner in which the bundle is attached to the wall is unknown. The bundle could be attached to the cell membrane, or physically anchored to the stiff peptidoglycan (PG) layer. These attachments will result in different interactions between the cell body and crescentin. We model the crescentin bundle as an elastic filament with bending rigidity a_0 and twist rigidity c_0. The configuration of the bundle, $\boldsymbol{u}$, can be described by a set of body-fixed coordinate frames along the length of the filament, $[\mathbf{e}^f{}_1, \mathbf{e}^f{}_2, \mathbf{e}^f{}_3]$. From these frames, one can compute the curvature and twist of the bundle. The filament bundle may also have intrinsic (preferred) curvature and twist (see Supporting Material).

Fig. 1 shows how forces may be transmitted from the cell body to the bundle: if the bundle is directly anchored to the cell wall, then the attachment requires that $\mathbf{e}^f{}_2 = \boldsymbol{n}'$ everywhere along the filament. If the bundle is anchored to the inner membrane, then this requirement is unlikely to be met, due to the soft and liquid nature of the plasma membrane. We can explore the shapes obtained by these modes of attachment by performing a mechanical calculation.

To find the mechanical equilibrium configurations of the cell, all forces on the cell body and the crescentin bundle must balance. The total elastic energy of the cell is

$$E = E_b + E_s + E_p + E_f, \tag{3}$$

where E_b and E_s are the bending and stretching energies of the cell wall; E_p is the work done by the turgor pressure; and E_f is the bending, twisting, and stretching energy of the crescentin bundle. The detailed forms of these energies are straightforward and are discussed in the Supporting Material and elsewhere (9). To obtain the current cell shape and the shape of the bundle, we minimize the overall energy with respect to parameters specifying the current shape and the bending and twist deformations of the crescentin bundle, $\boldsymbol{u}$. The approach here can be considered as a continuum version of a molecular model of the PG layer (10).

Fig. 2 shows several computed shapes for typical mature cell lengths (4–6 μm). We assume that the crescentin bundle has spontaneous (preferred) curvature and twist, and is a helix in absence of external forces. The intrinsic pitch of the bundle is ~1 μm and the bending stiffness is given by Eq. 2. The twist stiffness of the bundle is assumed to be ~3 times the bending stiffness. The cell body is assumed to be a straight ellipsoid cylinder in the absence of the crescentin bundle. Two different modes of attachment discussed in Fig. 1 are examined. For normal cells, we see that both attachment scenarios produce crescentlike shapes. If the bundle is rigidly anchored to the cell wall (Fig. 1 *B*), there is an overall slight twist in the cell body. Nevertheless, this shows, for the normal length cells, that two modes of attachment are in general indistinguishable.

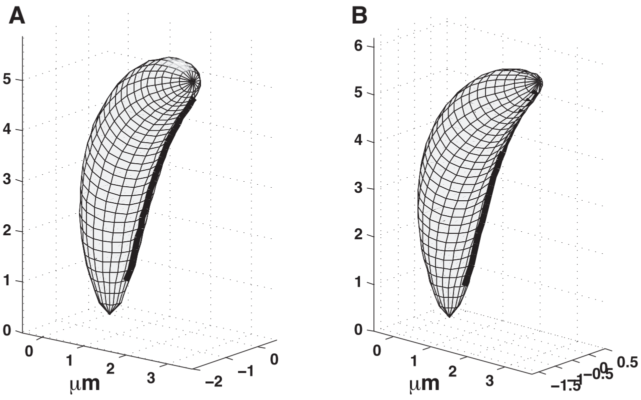

FIGURE 2 Typical shapes for normal-length cells. (*A*) The bundle is not physically anchored to the cell wall, and is attached in the fashion of Fig. 1 *C*. (*B*) The bundle is anchored to the cell wall (Fig. 1 *B*). There is a slight twist in the cell body. The dimensions of the axes are in microns.

The situation changes, however, when the cell body is longer, such as those during starved conditions. Different modes of attachment result in morphologically different cells (Fig. 3). To obtain helical cells, the bundle must be rigidly anchored to the cell wall (Fig. 1 *B*). If the bundle is attached to the membrane only, for all values of spontaneous curvature and twist of the bundle, the cell can only adopt circular shapes. Mechanically, to generate a helical cell, the torsion of the bundle must be coupled to the cell body. Thus, mechanics alone rules out an attachment model such as Fig. 1 *C*.

Further considerations show that the attachment mode of Fig. 1 *B* is also unlikely. To achieve such an attachment, the bundle must be twisted first and then anchored to the PG layer. The result is that the twist of the bundle in the cell must be different from the bundle spontaneous twist. (If the spontaneous twist is zero, helical cells are also not possible.) Therefore, work must be done to anchor the crescentin bundle to the cell wall. The work is proportional to the overall twist elastic energy in the bundle, which is estimated to be ~1×10^7 pN·nm. Another motor protein must expend energy to perform this work, and such a protein has not been found. Considering that crescentin is likely made of bundles of short coiled-coils and is mechanically soft, the cell shape is not likely the result of mechanical forces from crescentin.

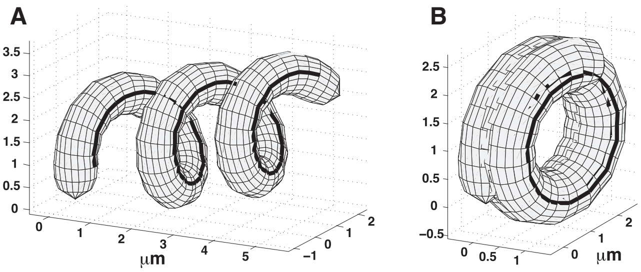

FIGURE 3 Longer cells with a stiff crescentin bundle. (*A*) The bundle is anchored to the cell as in Fig. 1 *B*. The pitch of the cell is ~2.9 μm. The crescentin bundle is twisted with respect to its equilibrium shape. (*B*) If the torsion of the bundle is not coupled to the cell wall, the cell never adopts a helical shape.

An alternative scenario is to view crescentin as a catalyst that alters the local growth mechanism of the PG layer, similar to the suggested role of MreB in the formation of rodlike bacteria (11). PG cell wall growth and remodeling is a complex multistep process (12); physical forces and chemical environments can alter PG growth rates. The presence of crescentin at one side of the cell can alter the local growth mechanism. Crescentin could also target growth enzymes to specific locations in the cell such that growth occurs asymmetrically, and/or influence the properties of the PG strands as they are inserted into the cell wall (13). Further studies of PG growth patterns in *C. crescentus*, interactions of crescentin with PG synthesis proteins, and how crescentin changes the peptidoglycan synthesis rates, will shed more light on its role in determining the cell shape.

SUPPORTING MATERIAL

Three tables and a figure are available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(09)00510-4.

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