Curvature and shape determination of growing bacteria

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Bacterial cells come in a variety of shapes, determined by the stress-bearing cell wall. Though many molecular details about the cell wall are known, our understanding of how a particular shape is produced during cell growth is at its infancy. Experiments on curved *Escherichia coli* grown in microtraps, and on naturally curved *Caulobacter crescentus*, reveal different modes of growth: one preserving arc length and the other preserving radius of curvature. We present a simple model for curved cell growth that relates these two growth modes to distinct but related growth rules—"hooplike growth" and "self-similar growth"—and discuss the implications for microscopic growth mechanisms.

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Bacteria display a wide variety of cell morphologies from spheres, rods, and helices to branched, tapered, and flat shapes [1]. In recent years, cell shape has been shown to play a critical role in regulating many important biological functions including nutrient access, cell division, attachment, dispersal, motility, predation, and cellular differentiation [2]. For most bacteria, the primary stress-bearing and shape-maintaining element is the cell wall: a meshwork of glycan strands cross-linked by peptide bridges [3]. In order to achieve cell growth, this peptidoglycan network must continuously reorganize. Though much is known about peptidoglycan and its enzymes, our understanding of how bacteria control cell-wall synthesis and hydrolysis in order to produce and maintain a particular shape is in its infancy. For example, in vibrioid (curved rod) or helical bacteria, the mechanisms for the control of cellular curvature are largely unresolved. For the crescent-shaped bacterium, *Caulobacter crescentus*, the intermediate filament homolog crescentin, which forms a single filamentous structure localizing to the inner cell curvature, is essential for curved cell growth [4]. Disruption of crescentin results in slow growth-dependent cell straightening [5]. Filamentous growth of *Caulobacter* produces spirals of fixed radius of curvature, dependent on crescentin concentration [5]. Recent experiments demonstrate that filamentous *Escherichia coli* cells, which would be straight in the absence of external constraints, also adopt a stable curved morphology when grown in circular agarose microchambers [6]. However, when released from their microchambers into growth media, the *E. coli* grow such that the trajectory of the cell axis expands uniformly while retaining its geometrical shape, i.e., the cells increase their radii of curvature. What do these two different modes of macroscopic growth teach us about the microscopic mechanisms of cell-wall synthesis? Here, we present a simple model for curved cell growth that relates the distinct behaviors of *Caulobacter* and *E. coli* to distinct growth rules.

As a first step toward developing a quantitative model for the growth of curved cells, it is important to determine the rules for cell growth in the absence of constraining forces or curvature-inducing cellular components such as crescentin. Hence, we first analyze the case of curved filamentous *E. coli* released from a microchamber into growth media; as discussed later, our analysis also applies to the growth of *Caulobacter* in the absence of crescentin. Upon release from confinement, the axis of an *E. coli* cell is typically a circular arc. Ignoring the relatively inert polar caps, we treat the cell wall as part of a toroidal surface (for longer cells the surface instead becomes helical). Since this shape is stable in the absence of external forces, the shape must be built into the structure of the cell wall. Call the radius of the arc r_0 and the subtended angle θ_0 (see Fig. 1), thus, the cell's total axis length is $\bar{L}=r_0\theta_0$. However, since the cell is curved, the length of the lateral cell wall is a function of the angle ϕ (see Fig. 2) about the cell's axis and is given by

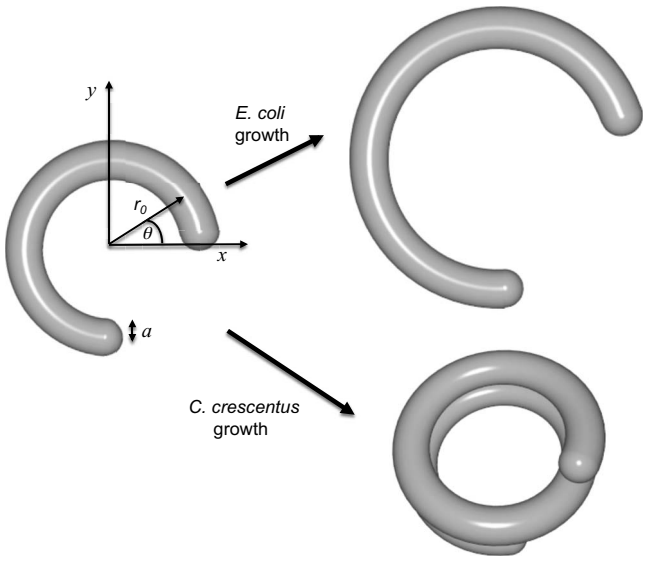

FIG. 1. Schematic of a curved cell showing the two observed modes of growth: (i) as in *Escherichia coli* where the angle subtended θ_0 (shown with $\theta_0 \approx 270^0$) remains a constant while the radius of curvature r_0 increases, and (ii) as in *Caulobacter crescentus* where θ_0 increases while the radius of curvature r_0 remains fixed during cell growth.

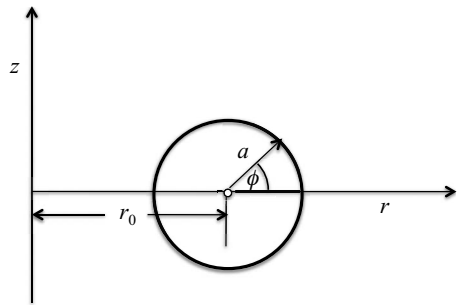

FIG. 2. Schematic of the cross-section of a toroidal cell. The radial distance r to the toroidal surface from the origin (i.e., the point at the global center of the torus) is a function of the angle ϕ. The total length of the cell wall for any ϕ is given by $L(\phi) = r(\phi)\theta_0$.

$$L(\phi) = (r_0 + a\cos\phi)\theta_0, \tag{1}$$

where a is the radius of the cell. As *E. coli* grow, it is observed that the radius of curvature r_0 increases while θ_0 remains approximately fixed [6]. What does this observation imply about the insertion of new cell wall? We find that the macroscopic growth of *E. coli* shown in Fig. 1 is consistent with "hooplike growth" in which complete hoops or rings of new material are inserted in the cell wall, perpendicular to the cell axis. Mathematically, hooplike growth implies that new cell wall is laid down at a fixed rate, independent of ϕ, so that after a time t the length of the cell wall increases to

$$L(\phi,t) = L(\phi) + \Delta L = [(1+\lambda)r_0 + a\cos\phi]\theta_0, \tag{2}$$

where $\lambda \equiv \Delta L/\bar{L}$ is independent of ϕ. The arc-radius after time t is then $(1+\alpha)r_0$, while the angle subtended remains θ_0, exactly as observed experimentally (cf. Fig. 1).

In contrast, we find that the observed mode of growth of filamentous C. crescentus, in which the cell's radius of curvature remains fixed, corresponds to a distinct rule for cell-wall insertion, which we term "self-similar growth." Put simply, self-similar growth means that cell wall is inserted locally in proportion to the amount of cell wall already present, with the experimental constraint that growth occurs only in the axial direction, with the cell radius a remaining fixed. Mathematically, during self-similar growth, the growth rate for each ϕ is proportional to $L(\phi,t)$, such that after a time t one finds

$$L(\phi,t) = L(\phi) + \Delta L(\phi) = [r_0 + a\cos\phi](1+\lambda')\theta_0, \tag{3}$$

where $\lambda' = \Delta L(\phi)/L(\phi)$ is independent of ϕ. In this case, as the cell grows, the curvature remains fixed while the angle subtended increases. This self-similar mode of growth describes the behavior of *Caulobacter* grown filamentously in the presence of crescentin (cf. Fig. 1).

What do these observations suggest about the underlying molecular mechanisms for cell-wall growth? For *E. coli*'s "hooplike growth," the rate at which new cell-wall material is laid down for all ϕ is proportional to the axis length of the cell and not the actual ϕ-dependent length. Such a growth mode strongly indicates that the cell employs a nonlocal mechanism to establish cell shape during growth, since insertion of a complete hoop of material requires a mechanism that spans the diameter of the cell. Even if complete hoops are not inserted, the insertion on average of the same amount of material across the diameter of the cell requires a cellular structure extending at least over this distance, roughly 1 micron in *E. coli*. In contrast, a purely local growth mechanism, for example, where new peptidoglycan is equally likely to be laid down everywhere along the lateral surface, such that the rate of growth of a cell-wall patch is proportional to its area, will instead generate self-similar growth (provided growth is constrained to occur only in the axial direction). Our analysis for *E. coli* therefore argues against a purely local growth rule and suggests the presence of a global structure directing growth. This is consistent with evidence that cytoskeletal structures such as helices of MreB and its homologs direct cell-wall growth in rod-shaped bacteria such as *E. coli* [7,8].

However, the observed macroscopic mode of growth of *Caulobacter* does not necessarily imply a purely local microscopic growth rule. Rather, the remarkable similarity of the macroscopic growth of crescentin-less *Caulobacter* [5] to that of curved filamentous *E. coli* upon release from the microtraps, suggests the operation of a global guide (>1 micron scale) to growth in *Caulobacter* as well. The observed similarity to *E. coli* implies that for crescentin-less *Caulobacter*, new cell wall is laid down at a rate that is approximately ϕ independent. The presence of crescentin clearly modifies the ϕ dependence of growth according to our analysis above. We therefore suggest two possibilities for the mechanism by which crescentin affects growth, (i) local, and (ii) long-range. In either case, in our view, the presence of crescentin perturbs a global hoop-like-growth mode, and the result mimics true self-similar insertion of new cell wall. The first possibility corresponds to disruption of peptidoglycan growth by crescentin filaments, perhaps via direct interaction with the protein complexes involved in peptidoglycan synthesis. We suggest testing this scenario via two-color dynamical *in vivo* imaging of fluorescently labeled cell-wall-synthesis enzymes and labeled crescentin to look for evidence of direct interactions. In the limit of a small number of fluorescently labeled proteins, *in vivo* tracking of single cell-wall-synthesis complexes, both in the presence and absence of crescentin, may also provide evidence for direct interactions. Alternatively, growth mismatch between the crescentin filament and the cell wall could generate a persistent ϕ-dependent stress distribution, with peptidoglycan deposition depending on local stress, as proposed in [5]. Studies of *Caulobacter* cell shape change under a sudden change in osmotic pressure or disruption of the crescentin filament, combined with detailed physical modeling (see, e.g., Ref. [9]), should reveal the magnitude of the mechanical stress due to crescentin and thus test the viability of the second scenario. More generally, we expect that studies of peptidoglycan growth patterns under a variety of conditions will help to reveal the role of cytoskeletal elements, including crescentin, in determining bacterial cell shape.

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