

Bacterial Shape: Concave Coiled Coils Dispatch Curve *Caulobacter*

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Bacterial cells exhibit a wide variety of shapes. Recent results indicate that the characteristic crescent shape of *Caulobacter crescentus* depends upon an intermediate filament-like protein that localizes to the concave side of the cell.

Eukaryotic cells have three main types of cytoskeletal element: microtubules, composed of tubulin; microfilaments, composed of actin; and intermediate filaments, made up of proteins from a number of different families. A decade ago, the evolutionary origin of these cytoskeletal systems was unknown. Now, a great deal of structural and biochemical evidence points to FtsZ and MreB as being the prokaryotic ancestors of tubulin and actin, respectively [1,2]. This suggests, not only that eukaryotic cytoskeletal proteins arose early in evolution, but also that bacteria have a cytoskeleton.

Whereas microtubules are involved in locomotion, mitosis and some aspects of cytokinesis, FtsZ seems to be limited to cytokinesis in prokaryotes and some eukaryotic organelles [3,4]. Actin is involved in many processes, including locomotion, cell growth, and cytokinesis, whereas MreB proteins function in controlling cell growth and shape, and have an as yet unclear role in chromosome segregation [5–7]. The cell shape function of the MreB family of proteins is striking. For example, inactivating a MreB homolog in normally cylindrical bacteria, such as *Escherichia coli* or *Bacillus subtilis*, causes the cells to lose their cylindrical shape and become the ‘default’ round shape [8,9].

Consistent with the view that MreB plays a role in imposing a more complex cylindrical cell architecture, naturally round bacteria such as streptococci do not have *mreB* homologs in their genomes. Interestingly, rhizobia and corynebacteria do not have MreB, but are still cylindrically shaped, possibly because they grow at their tips like filamentous fungi [5]. Their rod shape may depend on other proteins that are yet to be discovered.

If MreB proteins are required for maintaining a cylindrical shape in most bacteria, then what might confer what appear to be more complex shapes, such as helices? For example, vibrioid (‘comma’) or helical shapes are characteristic of a relatively small number of bacterial species. Is there a gene to keep a helical shape from reverting to a straight cylinder, analogous to the need for *mreB* in cylindrical shape? Or is helical shape the default, with specific genes being required for straight cylinders? Ausmees *et al.* [10] have recently addressed this question, and arrived at an astonishing answer.

Caulobacter crescentus is a popular model prokaryote, mainly because of its eukaryotic-like cell cycle control, different cell types and asymmetric growth [11]. A member of the prosthecate family of alpha-proteobacteria, *C. crescentus* derives its species name from the crescent shape of the cells, which have a flagellum at one pole and a stalk at the opposite pole. Interestingly, after a long time in stationary growth phase, *C. crescentus* can elongate into long, helical cells [12], suggesting that the crescent shape of the normal short cells is actually a truncated helix. The important breakthrough came when Ausmees *et al.* [10] found that a transposon insertion mutant caused *C. crescentus* cells to become straight rods. These rods still had stalks at one pole, indicating that their characteristic polarity was intact, but the curved shape was lost.

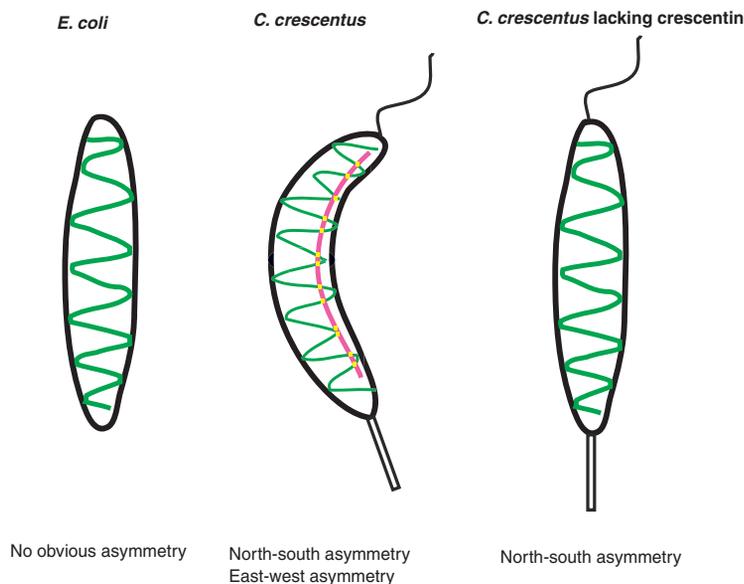
The authors then identified the protein encoded by the interrupted gene, which they dubbed CreS, for crescentin. Remarkably, studies with immunofluorescence or with a green fluorescent protein (GFP) fusion to CreS showed that crescentin localizes specifically to the concave surface of the cell, near the membrane. This localization pattern is continuous and not punctate, suggesting that the protein assembles into a polymer along the membrane, similar to FtsZ or MreB. This was supported by the observation that CreS–GFP localizes to a long continuous helix in stationary phase helical cells that matches the helical shape of the filamentous cells, hugging the inside curve.

The expression of CreS–GFP in the absence of wild-type CreS had a dominant-negative effect, ablating the usual curved cell shape. Interestingly, CreS–GFP under these conditions still localized to a helix within the straight cells, but this helix appeared to be detached from the membrane. This suggests that crescentin itself assembles into an intrinsic helical polymer and is not a decoration of an independent helical structure. Because a low copy plasmid expressing *creS* induced curved shape in the *creS* transposon insertion strain, the CreS filament must be able to form *de novo*, and is not dependent on continuation of existing crescentin structures.

Just these localization and function results would be of significant interest, but Ausmees *et al.* [10] did not stop there. A search for proteins with similar sequence to crescentin revealed weak homology with proteins having a particular organization of coiled-coil domains, one example of which is nuclear lamin A, a member of the intermediate filament protein family. The domain organization of crescentin is also similar to that of intermediate filament proteins, so the authors decided to test whether purified crescentin might polymerize under conditions used to assemble intermediate filaments. They found that crescentin can self-assemble into 10 nm wide filaments after solubilization in 6 M guanidinium and subsequent removal of the denaturant by dialysis, precisely the conditions used to assemble other intermediate filaments.

Figure 1. A model for crescentin action.

Crescentin localizes as an intermediate filament-like polymer bundle along the concave side of *C. crescentus* cells and is required for the characteristic curved shape of this species. It is proposed that the crescentin filament (red) interacts with the actin-like MreB coil (green) to inhibit cell wall biosynthesis at specific sites (yellow), resulting in left-right asymmetry during growth. Cells lacking crescentin, such as *E. coli* or crescentin-deficient *C. crescentus* mutants, are straight cylinders because of the action of MreB. Even without crescentin, *C. crescentus* displays one axis of cellular asymmetry, with a flagellum at one pole (top) and a stalk at the opposite pole (bottom).



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Intermediate filaments are present in many eukaryotic cells, but are not required in every cell type. Moreover, obvious homologs have not been found in plants and fungi, although intermediate filaments may still be present in these organisms. The roles of intermediate filaments are not completely understood, but they seem to provide resistance to mechanical stresses, and they can withstand deforming forces without breaking much better than can actin filaments or microtubules [13].

The nuclear lamins are thought to be the evolutionary precursors of the general intermediate filament superfamily [14]: they function as tensegrity elements, resisting deformation and conferring a specific shape on the nucleus [15]. Interestingly, a specific lamin is necessary to maintain hook-shaped nuclei in mouse spermatocytes and sufficient to induce hook-shaped nuclei in somatic cells [16]; this is intriguingly reminiscent of curved *C. crescentus* cells, although the mechanism is likely to be different. Lamin mutations result in shape abnormalities, even massive deformation, of the nuclear envelope. Lamins have also been implicated in transcriptional regulation [15]. So it is not far-fetched to think that crescentin-type proteins might be able to regulate cell shape in multiple ways, and even control genes and proteins involved in cell wall biosynthesis.

How might crescentin actually function at the molecular level in curving the bacterial cell? The requirement of crescentin for cell shape, and the nature of bacterial cell wall growth, suggest that crescentin cannot act simply as a stress fiber. *C. crescentus* must acquire its shape by synthesizing less net cell wall on the concave side of the cell than the convex side. It is likely, then, that crescentin interacts with the MreB cytoskeleton and other factors that govern cell wall synthesis (Figure 1). This hypothetical interaction between cytoskeletal elements is reasonable to propose, because cytoplasmic intermediate filament proteins of metazoans interact with actin and tubulin. For example, vimentin interacts with microtubules and slides along them [17]. Furthermore, alteration in the

structure or expression of bacterial actin homologs sometimes results in dramatically curved shapes in normally straight *E. coli* and *B. subtilis* cells [8,18], indicating that crescentin may mimic some of these abnormal interactions. Whether crescentin, like lamins, can induce shape changes in heterologous bacterial hosts is yet to be determined, but might be expected if the interacting partners are conserved.

How widespread are intermediate filament-like shape-determining proteins in bacteria? Standard sequence similarity searches are not too helpful, as they yield other proteins with coiled-coil domains, such as myosins. Ausmees *et al.* [10] did, however, find several candidate proteins in *Helicobacter pylori*, another curved bacterium, by searching for proteins with similar domain organization. It will be useful to learn whether vibrios, curved relatives of *E. coli*, have crescentin homologs. Interestingly, treponemes, members of the spirochete family, already have a well-known shape-determining cytoskeleton — a thick, helical cytoplasmic filament that is necessary for the distinctive helical shape of these organisms [19]. The filament itself is composed of only one protein, CfpA, which has no homologs in the database, but is in a complex that is anchored to the membrane and has other components that bridge and space the filaments [19,20]. As with crescentin, it is not known how CfpA exerts its cytoskeletal function. It is probable, however, that the crescentin filament, like the CfpA filament, will have many interacting partners. The big challenges now will be to find out how crescentin is directed to assemble asymmetrically on the concave side of the cell and how its putative interactions with other proteins translate into distinctive cellular architecture. Surely the answers lie just around the next curve.

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