Published in final edited form as:

Trends Microbiol. 2017 August ; 25(8): 673–687. doi:10.1016/j.tim.2017.03.006.

Shapeshifting to survive: shape determination and regulation in *Caulobacter crescentus*

Selamawit Abi Woldemeskel and Erin D. Goley*

Department of Biological Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Abstract

Bacterial cell shape is a genetically encoded and inherited feature that is optimized for efficient growth, survival and propagation of bacteria. In addition, bacterial cell morphology is adaptable to changes in environmental conditions. Work in recent years has demonstrated that individual features of cell shape, such as length or curvature, arise through the spatial regulation of cell wall synthesis by cytoskeletal proteins. However, the mechanisms by which these different morphogenetic factors are coordinated and how they may be globally regulated in response to cell cycle and environmental cues are only beginning to emerge. Here, we have summarized recent advances that have been made to understand morphology in the dimorphic Gram-negative bacterium, *Caulobacter crescentus*.

Keywords

Caulobacter crescentus; peptidoglycan; cytoskeleton; cell shape; stress response

The study of bacterial shape: why and how?

A survey of bacterial morphologies reveals a stunning array of diverse cell shapes, from simple rods and spheres to much more elaborate morphologies like stars, helices, or branched filaments [1,2]. For a given bacterial species, the shape of each cell is maintained with remarkable uniformity, but is adaptable to changes in environmental conditions or in response to developmental cues. Perhaps unsurprisingly, bacterial morphology is important for fitness, and is required for optimal growth and survival. Research into the mechanisms of bacterial cell shape over the last several decades has provided an increasingly clear picture of how particular aspects of cell shape are specified. The standard approach has been to take a specific feature of cell shape (e.g. cell width), identify the determinants of that feature using genetics or pharmacological approaches, and investigate the molecular mechanisms through which they act using biochemical, imaging, and/or biophysical approaches. Through this research, a paradigm has emerged wherein cytoskeletal proteins spatially regulate

*correspondence: egoley1@jhmi.edu (E.D. Goley).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

synthesis and remodeling of the bacterial cell wall to produce a particular shape attribute [3,4]. However, the mechanisms by which cytoskeletal proteins regulate cell wall synthesis are mostly unclear, and we are left with a larger question: how are the cell shape features that contribute to overall morphology regulated and coordinated as a cell progresses through the cell cycle and responds to environmental or developmental cues? Here we provide an overview of how the vibrioid α -proteobacterium, *Caulobacter crescentus*, adapts its shape as it progresses through the cell cycle and in the face of different environmental challenges. We highlight recent progress in understanding the molecular mechanisms of shape determination and emerging ideas of how shape may be regulated from a more global perspective.

***Caulobacter crescentus*: a model to investigate bacterial cell shape**

Caulobacter crescentus (referred to as *Caulobacter*) is a Gram-negative α -proteobacterium that inhabits nutrient-poor aquatic environments, a lifestyle facilitated by its dimorphic developmental cycle and curved-rod morphology. *Caulobacter* undergoes distinct morphological transitions as it progresses through its cell cycle (Fig. 1A), making it an excellent model system for addressing cell shape specification and regulation [5,6]. Cell division in *Caulobacter* gives rise to two dissimilar daughters: a motile swarmer cell and a sessile stalked cell. The swarmer cell has a flagellum and adhesive pili that protrude from a single pole and is unable to initiate DNA replication or progress through the cell cycle. In order to divide, the swarmer cell must first differentiate into a stalked cell, which includes a morphological transition characterized by ejection of its flagellum, retraction of its pili, and growth of a cylindrical extension of the cell envelope called a stalk at the same pole (Fig. 1A). The stalked cell elongates, replicates its DNA and grows a flagellum at the pole opposite the stalk, giving rise to a pre-divisional cell. Finally, cell compartmentalization and separation yields two biochemically and morphologically distinct daughter cells: a stalked cell that can immediately enter another round of the cell cycle and a swarmer cell that must differentiate into a stalked cell before being able to divide.

Studies of morphological changes that occur over the *Caulobacter* cell cycle are enabled by a simple synchronization procedure that yields a relatively pure population of swarmer cells [7]. Moreover, *Caulobacter* morphology is altered upon starvation (e.g. [8]) or entry into stationary phase [9], providing an opportunity to study the regulation of cell shape in response to environmental cues. In the next sections, we describe our current understanding of the molecular mechanisms of shape determination in *Caulobacter*, then move on to global regulation of cell morphology.

The great wall: peptidoglycan is a primary determinant of bacterial cell shape

In *Caulobacter*, as in almost all bacteria, the peptidoglycan (PG) cell wall is both necessary and sufficient to maintain cell shape. The cell wall resides just outside the cytoplasmic membrane and is one large, covalently-linked molecule of glycan strands crosslinked by short peptides [4]. Its primary function is to confer mechanical integrity to the cell envelope so that it can withstand turgor pressure without lysis. For the cell to grow, divide, or change

its morphology, PG must be remodeled through the coordinated action of dozens of enzymes. In the first stage of PG synthesis, disaccharide pentapeptide subunits are produced in the cytoplasm as lipid-linked precursors and flipped across the cytoplasmic membrane [10,11]. Glycosyltransferases then polymerize the disaccharide subunits into a nascent PG chain while transpeptidases crosslink the peptide moieties to form a meshwork. PG hydrolases work in concert with cell wall synthases to break pre-existing bonds, allowing insertion of new material, removal of old material, and maintenance of a cell wall of appropriate mechanical properties [12]. Synthetic and hydrolytic activities must be precisely coordinated so that cell wall integrity is not compromised. The mechanisms by which bacteria synthesize and modify the cell wall to give rise to different shapes are the subject of active investigation, but it is evident that PG enzymes must be spatially regulated for the specification of cell shape.

Regulating shape from the inside out: cytoskeletal proteins as shape determinants

If PG synthesis were not spatially regulated, the only shape that could be achieved is a sphere that grows isotropically and never divides. Work in recent years has demonstrated that cytoskeletal proteins play a central role in spatially regulating PG synthesis and contributing to the diversity of shapes observed in nature. In this section, we describe the players for defining width, length, curvature, and stalk morphology in *Caulobacter* (Table 1).

Making a rod

The actin homolog, MreB, is essential for maintenance of cell width in many rod-shaped bacteria, including *Caulobacter*. Depleting or inactivating MreB in *Caulobacter* causes cells to increase in width, giving rise to lemon-shaped cells that ultimately lyse (Fig. 1B and [13,14]). Restoration of MreB function after cell rounding allows for recovery of rod shape, but requires a period of PG synthesis and comprises a series of morphological changes including cell thinning and elongation [15]. *In vitro*, MreB forms short, membrane-associated, antiparallel double filaments [16]. In *Caulobacter* cells, MreB localization is cell cycle-dependent: MreB is distributed in patches along the length of swarmer cells, is recruited to midcell in stalked cells, and becomes patchy and dispersed again before cytokinesis is completed (Fig. 1A and [13,14,17]). Midcell accumulation of MreB requires the cell division GTPase, FtsZ [18], but it is not clear how FtsZ recruits MreB nor what triggers redistribution of MreB prior to cytokinesis. Work in *Escherichia coli* suggests that MreB and FtsZ may directly interact, but whether the same is true in *Caulobacter* remains to be established [19]. One binding partner of MreB, MbiA, has been identified that, when overproduced, promotes midcell accumulation of MreB and cell rounding [20]. *mbiA* is non-essential, however, and MreB localization and cell morphology are normal in its absence. Moreover, the importance of the cell cycle-dependent localization changes of MreB for morphology maintenance is not clear, as mutants of MreB with near-wildtype morphologies but no midcell localization have been isolated [18,21].

MreB cooperates with a large cohort of morphogenetic proteins to exert its effects on PG metabolism and cell shape (Table 1). Extensive genetic, imaging, two-hybrid, and

biochemical analyses suggest a complex network of interactions linking MreB to PG metabolic enzymes both for precursor synthesis and PG polymerization and crosslinking [14,18,22–26]. MreB is required for the localized assembly of at least a subset of these PG metabolic and morphogenetic proteins, leading to a model wherein MreB's primary role is that of a scaffold that locally promotes PG synthesis. In *E. coli*, membrane-associated MreB localizes preferentially to regions of local negative curvature, or indentations, of the membrane. It then recruits the PG metabolic machinery and promotes local PG synthesis in these areas, correcting deviations from rod shape [27]. In *E. coli* and *Bacillus subtilis*, MreB is distributed in patches that move circumferentially around the cell in a manner that is dependent on PG synthesis [28–30] and on the MreB-interacting protein, RodZ [31]. Circumferential movement of MreB is proposed to evenly distribute PG synthesis during growth. Though not strictly required to maintain rod shape in *E. coli* when cells are grown in rich media, circumferential movement is required for rod shape during periods of cell wall stress [31]. In light of the conservation of MreB and most of the morphogenetic proteins involved in rod shape, it is likely that the mechanisms described above for *E. coli* and *B. subtilis* are also at play during maintenance of cell width and rod shape in *Caulobacter*.

Curving a rod

Unlike *B. subtilis* and *E. coli*, *Caulobacter* cells have a gentle curvature that is dependent on the intermediate filament-like protein, crescentin [32]. Crescentin forms a filamentous structure that is attached to the cytoplasmic membrane at the inner curvature of the cell (Fig. 1A). Crescentin, encoded by *creS*, is not essential, and cells with a *creS* deletion grow as straight rods (Fig. 1B and [27]). Loss of crescentin does not result in an immediate loss of curvature, however: a cell must remodel its PG in the absence of crescentin for straight cell morphology to emerge [33]. It is proposed that crescentin induces curvature by exerting a compressive force on the cell wall at the inner curvature [33]. Crescentin-imposed force is hypothesized to reduce strain in peptide bonds in the cell wall proximal to crescentin and make insertion of new glycan strands less favorable. This promotes a gradient of PG synthesis across the short axis of the cell, leading to cell curvature as more PG is inserted on the crescentin-distal side than the crescentin-proximal side. This mechanism of crescentin-induced curvature requires both membrane association and polymerization of crescentin [33–35].

The precise mechanism by which crescentin associates with the membrane is not clear, but it seems to depend on MreB. Disrupting MreB by treating cells with the MreB inhibitor, A22, disrupts crescentin membrane association [21,35]. Similarly, expressing certain A22-resistant mutants of MreB in place of wild type also disrupts crescentin localization and affects cell curvature without affecting cell width [21,35]. Thus, MreB has independent roles in defining cell width and curvature, and may affect curvature by promoting crescentin anchoring at the membrane. A third polymeric protein, the metabolic enzyme CTP synthase, plays a crescentin-dependent role in defining cell curvature, but appears to oppose the action of crescentin. Increasing CTP synthase levels leads to cell straightening, while depleting it yields hypercurvature [36].

Interestingly, *Caulobacter* cell curvature does not confer a growth advantage in planktonic cultures in the laboratory [32]. However, when *Caulobacter* cells are grown on a surface under moderate flow to more closely model its natural environment, crescentin-dependent cell curvature significantly enhances surface colonization [37]. Stalked and pre-divisional *Caulobacter* cells attach irreversibly to environmental surfaces using a holdfast, an adhesive polysaccharide secreted at the tips of their stalks. It is proposed that under flow, the new pole of a surface-attached, curved pre-divisional cell would contact the adjacent surface, triggering transient attachment via its adhesive pili. Upon cell separation and differentiation to a stalked cell, the daughter cell would then irreversibly attach to the nearby surface. For a straight pre-divisional cell, the new pole is less likely to reach the adjacent surface under moderate flow, making it more likely that the newborn swarmer would disperse instead of colonizing nearby [32].

Dividing a rod

A third basic feature of *Caulobacter* cell shape is its length, which is defined by the timing and location of cell division. As for width and curvature, a cytoskeletal regulator of PG remodeling is central to division: in this case the broadly conserved, polymerizing GTPase, FtsZ [38]. In the absence of FtsZ, cells fail to divide, become elongated, and eventually lyse (Fig. 1B and [39,40]). FtsZ forms linear head-to-tail polymers *in vitro*, and assembles into a dynamic, discontinuous “Z-ring” that marks the incipient division site in the cell (Fig. 1A and [41–43]). Z-ring assembly is coordinated with chromosome segregation in time and space by a negative regulator of FtsZ polymerization called MipZ [41]. MipZ associates with the polar chromosomal centromeres and with the nucleoid, forming a gradient that extends from each pole after centromere segregation (Fig. 1A and [41,44]). The Z-ring assembles at the site of lowest MipZ concentration, near midcell, where it initiates assembly of the division machinery.

In order to facilitate cytokinesis, FtsZ recruits over a dozen proteins to midcell (Table 1 and [17,18,45–49]). Several of these factors interact directly with FtsZ and regulate its structure, dynamics, and/or membrane attachment [39,50,51]. Importantly, many proteins recruited downstream of FtsZ have enzymatic activity towards the cell wall, including the PG polymerase FtsW [17,52,53], the transpeptidase FtsI [48], and proteins implicated in PG hydrolysis [46,47,51,54]. In *Caulobacter*, FtsW is one of the last proteins recruited to the division site and its enrichment may trigger constriction [17,55,56]. Recent work in *Caulobacter* suggests that, in addition to simply recruiting proteins to the division site, FtsZ may actively regulate PG remodeling through a mechanism that requires its disordered C-terminal linker [57]. In addition, in both *B. subtilis* and *E. coli*, the rate of FtsZ movement *in vivo*, which is determined at least in part by its GTPase rate, was recently demonstrated to drive the rate or organization of PG metabolism [58,59]. FtsZ has long been proposed to generate a constrictive force [60,61] that could be used to mechanically regulate PG metabolism [62]. However, as there is no evidence that FtsZ generates meaningful force *in vivo*, the detailed mechanisms by which FtsZ interacts with and regulates PG metabolism remain to be determined.

Extending a stalk

In addition to the shape of its cell body, a hallmark of *Caulobacter* morphology is the polar stalk that is formed upon the swarmer-to-stalked cell transition (Fig. 1C). The stalk is about one fifth the diameter of the cell body, contains all layers of the cell envelope, and extends from the pole previously occupied by the flagellum. Though continuous with the cell body, *Caulobacter* stalks are compartmentalized and lack cytoplasmic proteins and DNA [63]. Diffusion of membrane and periplasmic proteins between the stalk and cell body is limited by proteinaceous cross-bands that periodically extend across the short axis of the stalk from outer membrane to outer membrane (Fig. 1C and [64,65]). Apart from acting as diffusion barriers, however, cross-bands do not seem to contribute to stalk growth or morphology [65].

Of the shape features we have discussed, we arguably know the least about how the shape and growth of the stalk is specified. No factor has been identified that is directly and specifically required for stalk synthesis. There are stalkless mutants of *Caulobacter*, but these tend to be upstream regulators with pleiotropic developmental defects [66–68]. We do know that stalk formation requires PG synthesis, with new cell wall being inserted at the base of the stalk near the cell body and pushed outward as the stalk extends [8,18]. The Brun lab proposed that stalk morphogenesis is a specialized form of cell elongation, as MreB, RodZ, and RodA, which are important for cell elongation and width maintenance, are required for normal stalk morphology [25,63,69]. In addition, a number of proteins have been identified that localize either to the base of the stalk or throughout the stalk and contribute to stalk elongation (Table 1). Two of these, BacA and BacB, are members of a bacteria-specific family of cytoskeletal proteins called bactofilins [70]. BacA and BacB assemble into filamentous ribbons *in vitro*, and form a membrane-bound sheet-like structure at the base of the stalk in cells [64]. Like other cytoskeletal regulators of cell shape, the bactofilins are proposed to influence stalk morphogenesis through cell wall remodeling. They are required for recruitment of the bifunctional penicillin binding protein, PbpC, to the base of the stalk [70]. PbpC, in turn, is required for stalk localization of StpX, a stalk protein that may play a role in metal homeostasis [71,72]. Though morphologically normal stalks form in the absence of each of these factors under nutrient replete conditions, stalks fail to elongate to the same extent as wildtype when mutant cells are starved for phosphate. The precise role each plays in promoting stalk elongation remains to be determined.

Cell cycle regulation of shape

Of the shape attributes described above, cell width and curvature remain relatively constant over the cell cycle while length and stalk morphogenesis are cell cycle regulated. In *Caulobacter*, cell cycle progression and the corresponding shape changes occur at specific times due to a robust regulatory circuit of transcriptional control layered with translational control, proteolysis, and/or phosphorylation of key factors [5,6]. This circuit affects the cell cycle-dependent expression and activity of hundreds of factors, including those required for morphogenesis.

In the case of cell division, we described above how Z-ring assembly is coordinated in time and space with chromosome segregation through the action of MipZ. Although MipZ levels are constant over the cell cycle [41], levels and activities of at least two regulators of

chromosome replication initiation, CtrA and DnaA, vary over time to permit chromosome replication and segregation only at the swarmer to stalked cell transition [5]. MipZ follows the segregating centromeres at that time to establish a bipolar gradient and promote Z-ring assembly near midcell. FtsZ protein levels are additionally cell cycle regulated through transcriptional regulation [73,74] and proteolysis [75,76]. FtsZ concentration is low in swarmers, increases to allow Z-ring formation in stalked cells, and peaks in pre-divisional cells prior to cytokinesis. Levels of other key division proteins are similarly cell cycle regulated to peak in pre-divisional cells [49,74,77,78]. Collectively, these mechanisms ensure that cell division may only occur near midcell in late pre-divisional cells that have segregated their chromosomes.

Though the mechanisms are incompletely understood, it is clear that stalk morphogenesis is subject to transcriptional, translational, and post-translational controls as well. Two key transcriptional regulators of stalk formation, TacA and StaR, have been identified that yield stalkless cells when deleted and grown in rich media [66,68]. While *staR* transcription is controlled by TacA, the levels of TacA are controlled by the master transcriptional regulator, CtrA, and by the ClpXP protease, such that TacA is most abundant in late pre-divisional cells [74,79,80]. TacA is further activated by a phosphorelay comprising the histidine kinase ShkA and histidine phosphotransferase ShpA [68], and is inhibited by the developmental regulator SpmY [81]. Levels of StaR, a transcriptional target of active TacA, are consequently highest in newborn swarmer cells and, presumably, StaR activates expression of key factors for stalk formation upon differentiation to a stalked cell fate [68]. These observations help to explain the timing of stalk synthesis during the swarmer-to-stalked cell transition. However, *tacA* and *staR* mutants can still be induced to make stalks when starved for phosphate [68], indicating that there are additional regulators of environmentally-induced stalk formation.

Shapeshifting during environmental stress

The freshwater environments *Caulobacter* naturally inhabits are often limiting for fundamental nutrients like carbon, nitrogen, and phosphate. *Caulobacter* has therefore devised ways to adapt its growth to changes in nutrient availability. This includes changing its morphology during phosphate starvation, stationary phase, and activation of the stringent response, as described in the following sections.

Phosphate starvation

When phosphate levels are low, *Caulobacter* cells undergo a distinct morphological transition, elongating their stalks to up to 30 microns (~10–15 cell bodies) in length (Fig. 2 and [8]). The cell bodies of phosphate-starved cells also increase in length and width, resulting in an ~2.5-fold increase in cell body area (Fig. 2 and [8,82]). Initially, it was hypothesized that stalk elongation during phosphate starvation would enhance phosphate absorption by increasing the surface area to volume ratio of the cell [83]. However, the diffusion barrier for periplasmic and membrane proteins created by the stalk cross-bands would inhibit movement of phosphate bound to its periplasmic carrier, PstS, from the stalk

to the cell body [65]. As such, phosphate taken up into the stalk would never make it to the cell body for cytoplasmic import [84].

An alternative hypothesis for the purpose of stalk elongation during phosphate starvation has recently been proposed. As described in the section on cell curvature, at the tip of the *Caulobacter* stalk is an adhesive glue called holdfast that the cell uses for irreversible attachment to surfaces [85,86]. When the stalk elongates during phosphate starvation, this would elevate the cell body away from the surface. Movement of the *Caulobacter* cell body further from the surface is proposed to allow *Caulobacter* cells to access nutrients in the water flowing above the surface to which they are attached, and provide a competitive advantage over other microorganisms that are attached close to the surface [37,84]. However, other stalked bacteria, for example *Asticcacaulis* species, have non-polar stalks and secrete holdfast at the cell pole instead of the stalk tip [8,87]. Though they also elongate their stalks in response to phosphate starvation, stalk elongation would not affect the distance of the bacterium from the environmental surface. A distinct role of stalk elongation in promoting survival must therefore exist in those organisms, and possibly, in *Caulobacter*.

While we are still considering why *Caulobacter* cells adapt their morphology in response to changes in phosphate availability, another important question is how do they do this? In the section on stalk morphology, we introduced the bactofilins, PbpC, and StpX as being important for elongation of the stalk in response to phosphate starvation, though the precise roles of those factors in that process are not known. It seems reasonable to propose, however, that PbpC, a cell wall polymerase and transpeptidase, might directly synthesize PG that is used to elongate the stalk. In addition, low phosphate levels signal the response regulator PhoB to initiate a transcriptional response that includes upregulation of dozens of genes [82,88]. Within the PhoB regulon are a handful of cell wall enzymes and many uncharacterized hypothetical proteins that could play important roles in stalk elongation [88], but these await experimental validation.

Stringent response

Like most bacteria, *Caulobacter* employs the small molecule alarmone, guanosine tetra- or pentaphosphate ((p)ppGpp) to respond to a variety of stress signals. *Caulobacter* possesses a single enzyme that both synthesizes and hydrolyses (p)ppGpp called SpoT [89]. Low levels of (p)ppGpp are maintained in cells during normal growth in the laboratory, but (p)ppGpp levels are elevated when cells are starved for carbon, nitrogen, or amino acids [89,90]. (p)ppGpp slows the swarmer-to-stalked transition during starvation, perhaps to allow the swarmer cell more time to seek out nutrients before differentiating into a stalked cell (Fig. 2 and [90–92]). (p)ppGpp exerts its effects on the cell cycle, at least in part, by affecting the levels of CtrA and DnaA, each of which directly impacts DNA replication initiation and regulates expression of dozens of genes required for cell cycle progression [92].

Interestingly, in addition to delaying swarmer cell differentiation, elevating (p)ppGpp levels in non-starved cells through the inducible expression of a constitutively active (p)ppGpp synthetase or by blocking lipid biosynthesis causes a reduction in cell size at all stages of the cell cycle (Fig. 2 and [92,93]). These observations suggest that (p)ppGpp ultimately regulates cell wall metabolism and are consistent with findings implicating (p)ppGpp in

conferring resistance to antibiotics that target the cell wall in *E. coli* [94]. Our understanding of the mechanisms by which (p)ppGpp regulates cell wall metabolism is complicated by the fact that (p)ppGpp broadly impacts the transcriptome and proteome by binding directly to the ribosome and to RNA polymerase, among other partners [95]. The specific targets of (p)ppGpp that mediate its effects on cell wall metabolism and cell size in *Caulobacter* therefore remain to be elucidated.

Stationary phase

A final environmental condition that significantly impacts *Caulobacter* morphology is prolonged culturing of cells in rich media [9]. Upon entry into stationary phase (p)ppGpp levels are elevated [96] and swarmer cells with high levels of CtrA and 1N DNA content accumulate in the first 24 hours [96,97]. However, coincident with a loss in viability as the stationary phase culture ages over several days, cells undergo “morphological adaptation”. They elongate, decrease in width, and become helical in shape (Fig. 2 and [9]). Proper morphology returns for at least some fraction of the population after diluting and growing cells in exponential phase for several doublings [84]. The purpose of these shape changes during late stationary phase are unclear, though stationary phase cells are more resistant to a variety of environmental stresses than cells growing in log phase. Interestingly, morphological adaptation to stationary phase is media-dependent, as cells similarly cultured in minimal medium do not undergo the morphological changes described above [9].

How does morphological adaptation to late stationary phase occur? The cell elongation observed during late stationary phase almost certainly results from a drop in FtsZ concentration [9]. The coiling of these cells into helices requires the presence of crescentin [32]. However, other treatments that promote cell elongation, for example genetic depletion of FtsZ, do not lead to formation of helical cells. Instead, cells grow as gently curved filaments [40]. This suggests that a mechanism must be in place to promote hypercurvature in stationary phase cells that is in addition to inhibition of division through reduced FtsZ levels. *Caulobacter* cells in stationary phase differentially regulate expression of many genes to promote survival and, perhaps, morphological changes through transcriptional regulators such as SpdR [98–100].

Shape rules: quantitative principles governing cell size and shape homeostasis

In recent years, several groups have sought to define quantitative rules that govern cell size homeostasis and are therefore relevant to any discussion of cell shape regulation. Two concepts have gained experimental support recently for regulating *Caulobacter* cell size and shape: (i) length homeostasis through “adder” or “mixer” mechanisms [101,102] and (ii) maintenance of a constant surface area to volume ratio under a given physiological condition [103,104]. The adder principle states that bacterial cells, including *Caulobacter*, achieve cell length homeostasis by growing a relatively constant amount prior to each cell division, regardless of length at birth [101]. This idea is supported by time-lapse observation of the growth of thousands of cells, and contradicts earlier models such as the “sizer” model in which cells grow to a particular cell length threshold before dividing. A related model has

been recently proposed, positing that *Caulobacter* cells grow according to a “mixer” mechanism (pre-print, [102]). Cells grow for a fixed fraction of their total cell cycle prior to initiating constriction (i.e. a “relative timer”), then convert to a “pure adder” mechanism during constriction, when they grow by a fixed length. This idea is supported by fitting observed growth behaviors with timer, adder, and mixer mathematical models. Moreover, a biphasic growth mechanism is consistent with the two known growth modes of *Caulobacter* driven by the elongation and division machineries before and after constriction initiation, respectively. Of course, molecular mechanisms must be in place to sense the relative passage of time and changes in cell length and, indeed, for the latter, cells may actually measure any parameter related to length such as cell mass, surface area, or volume [95].

The second principle recently defined for *Caulobacter* is maintenance of a constant surface area to volume ratio for a given growth condition [103,104]. Harris and Theriot observed that the growth rate of the cell surface scales with cell volume for a given growth condition. Changing growth conditions such that the ratio of surface synthesis to volume synthesis is altered resets cell size. For example, if surface synthesis increases relative to volume synthesis, cells become smaller on average and if surface synthesis decreases relative to volume synthesis, cells become larger. They hypothesized that synthesis of limiting cell surface materials in the cytoplasm links surface growth rate to cell volume [97]. Although the precise molecular components at play remain to be determined, the adder/mixer principles and surface area to volume ratio maintenance will help to guide emerging ideas for how *Caulobacter* cell shape and size are changed during environmental changes. The reduction of cell size upon (p)ppGpp production, for example, may reflect an increase in surface area synthesis relative to volume synthesis.

Concluding Remarks

Cell shape is such a critical determinant of fitness that even the simplest organisms go to great lengths to control it. *Caulobacter* is no exception, and significant effort over the last several decades has been focused on uncovering the mechanisms by which *Caulobacter* defines, maintains, and changes its shape. Through these studies, we have an extensive, and growing, list of the molecular players involved in executing specific aspects of cell shape, including width, length, curvature, or stalk formation (Table 1). In some cases, for example cell curvature, specific mechanisms have been proposed, and experimentally supported, for how cytoskeletal proteins interface with cell wall remodelers to achieve a particular morphological feature. For others, notably stalk formation, we know far less about how that aspect of shape develops. As our understanding of the mechanisms of shape grows, we are tasked with formulating a systems level view of how cells globally coordinate distinct aspects of cell shape during cell cycle progression and changes in growth conditions. We know that *Caulobacter* cells adapt their morphologies differentially during times of stress, and we have acquired lists of transcripts and proteins that change in levels and/or activity upon many of those changes in growth conditions. We have yet to cull these lists and experimentally validate putative mediators of shape change (see Outstanding Questions). Emerging ideas on quantitative principles governing cell size and shape homeostasis will inevitably inform and guide future models of shape specification, maintenance, and adaptation.

Outstanding Questions

- It is now the paradigm that cell shape in bacteria is determined through the regulation of cell wall synthesis in time and space by cytoskeletal proteins. However, fundamental mechanistic questions remain. What are the molecular and physical mechanisms by which cytoskeletal proteins direct cell wall remodeling? Do cytoskeletal proteins generate force to influence PG remodeling? If so, how? What are the physical connections between cytoplasmic cytoskeletal proteins and PG enzymes in the periplasm?
- Of the shape features described in *Caulobacter*, morphogenesis of the stalk is still relatively mysterious. Do stalks form through differential regulation of the existing morphogenetic machinery (e.g. MreB and the elongation machinery)? If so, how is the same machinery used to generate structures with starkly different dimensions? If not, why have we not identified any factors that are absolutely and specifically required for formation of a stalk?
- In the past, we have largely approached cell shape by breaking it into concrete shape features and assessing how each is specified. How do these numerous shape determinants work together to ensure proper overall morphology of the cell? What crosstalk is at play between length, width, and curvature determination? MreB, for example, seems to play roles in cell width, curvature, and stalk morphogenesis. What are the mechanisms by which it participates in multiple morphogenetic events?
- *Caulobacter* cell cycle control is famously regulated by master cell cycle transcriptional regulators. Are there global transcriptional regulators of cell shape that impact multiple shape attributes in a coordinated fashion? If so, what are their targets?
- Adaptation of bacterial physiology is central to survival under a variety of stresses, and we now appreciate many of the ways in which *Caulobacter* changes its morphology in response to starvation and other stresses. We have begun to identify upstream regulators of these shape changes, such as transcription factors and small molecules. Our understanding is far from complete, however. What are the downstream targets of these signaling cascades that ultimately impact cell shape? How do these stresses coordinately affect multiple aspects of cell shape? How do morphological changes provide fitness and survival advantages during stress?
- The field has begun to define the quantitative principles that guide bacterial cell shape homeostasis. The specific mechanisms by which these principles are followed are not known. What are the molecular and/or physical mechanisms underlying the adder and mixer principles, and the maintenance of surface area to volume ratio?

Acknowledgments

We wish to thank members of the Goley and Xiao laboratories for fruitful discussions about bacterial cell shape regulation. We are grateful to Elizabeth Meier and Kousik Sundararajan for feedback on this manuscript. Work in the Goley lab on bacterial cell shape is funded by the National Institutes of Health through award R01GM108640 (to EDG).

References

1. Kysela DT, et al. Diversity Takes Shape: Understanding the Mechanistic and Adaptive Basis of Bacterial Morphology. *PLoS Biol.* 2016; 14:e1002565. [PubMed: 27695035]
2. Young KD. Bacterial shape: two-dimensional questions and possibilities. *Annu Rev Microbiol.* 2010; 64:223–240. [PubMed: 20825347]
3. Cabeen MT, Jacobs-Wagner C. Skin and bones: the bacterial cytoskeleton, cell wall, and cell morphogenesis. *J Cell Biol.* 2007; 179:381–387. [PubMed: 17967949]
4. Typas A, et al. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nat Rev Microbiol.* 2012; 10:123–136.
5. Collier J. Cell cycle control in Alphaproteobacteria. *Curr Opin Microbiol.* 2016; 30:107–113. [PubMed: 26871482]
6. Lasker K, et al. An intracellular compass spatially coordinates cell cycle modules in *Caulobacter crescentus*. *Curr Opin Microbiol.* 2016; 33:131–139. [PubMed: 27517351]
7. Evinger M, Agabian N. Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. *J Bacteriol.* 1977; 132:294–301. [PubMed: 334726]
8. Schmidt JM, Stanier RY. The development of cellular stalks in bacteria. *J Cell Biol.* 1966; 28:423–436. [PubMed: 5960805]
9. Wortinger MA, et al. Morphological adaptation and inhibition of cell division during stationary phase in *Caulobacter crescentus*. *Molecular Microbiology.* 1998; 29:963–973. [PubMed: 9767565]
10. Sham LT, et al. Bacterial cell wall. MurJ is the flippase of lipid-linked precursors for peptidoglycan biogenesis. *Science.* 2014; 345:220–222. [PubMed: 25013077]
11. Meeske AJ, et al. MurJ and a novel lipid II flippase are required for cell wall biogenesis in *Bacillus subtilis*. *Proc Natl Acad Sci USA.* 2015; 112:6437–6442. [PubMed: 25918422]
12. Uehara T, Bernhardt TG. More than just lysins: peptidoglycan hydrolases tailor the cell wall. *Curr Opin Microbiol.* 2011; 14:698–703. [PubMed: 22055466]
13. Gitai Z, et al. An actin-like gene can determine cell polarity in bacteria. *Proc Natl Acad Sci USA.* 2004; 101:8643–8648. [PubMed: 15159537]
14. Figge RM, et al. MreB, the cell shape-determining bacterial actin homologue, co-ordinates cell wall morphogenesis in *Caulobacter crescentus*. *Molecular Microbiology.* 2004; 51:1321–1332. [PubMed: 14982627]
15. Takacs CN, et al. MreB drives de novo rod morphogenesis in *Caulobacter crescentus* via remodeling of the cell wall. *J Bacteriol.* 2010; 192:1671–1684. [PubMed: 20023035]
16. van den Ent F, et al. Bacterial actin MreB forms antiparallel double filaments. *eLife.* 2014; 3:e02634. [PubMed: 24843005]
17. Goley ED, et al. Assembly of the *Caulobacter* cell division machine. *Molecular Microbiology.* 2011; 80:1680–1698. [PubMed: 21542856]
18. Aaron M, et al. The tubulin homologue FtsZ contributes to cell elongation by guiding cell wall precursor synthesis in *Caulobacter crescentus*. *Molecular Microbiology.* 2007; 64:938–952. [PubMed: 17501919]
19. Fenton AK, Gerdes K. Direct interaction of FtsZ and MreB is required for septum synthesis and cell division in *Escherichia coli*. *EMBO J.* 2013; doi: 10.1038/emboj.2013.129
20. Yakhnina AA, Gitai Z. The small protein MbiA interacts with MreB and modulates cell shape in *Caulobacter crescentus*. *Molecular Microbiology.* 2012; 85:1090–1104. [PubMed: 22804814]
21. Dye NA, et al. Mutations in the nucleotide binding pocket of MreB can alter cell curvature and polar morphology in *Caulobacter*. *Molecular Microbiology.* 2011; 81:368–394. [PubMed: 21564339]

22. Dye NA, et al. Two independent spiral structures control cell shape in *Caulobacter*. *Proc Natl Acad Sci USA*. 2005; 102:18608–18613. [PubMed: 16344481]
23. Divakaruni AV, et al. The cell-shape protein MreC interacts with extracytoplasmic proteins including cell wall assembly complexes in *Caulobacter crescentus*. *Proc Natl Acad Sci USA*. 2005; 102:18602–18607. [PubMed: 16344480]
24. Divakaruni AV, et al. The cell shape proteins MreB and MreC control cell morphogenesis by positioning cell wall synthetic complexes. *Molecular Microbiology*. 2007; 66:174–188. [PubMed: 17880425]
25. Alyahya SA, et al. RodZ, a component of the bacterial core morphogenic apparatus. *Proc Natl Acad Sci USA*. 2009; 106:1239–1244. [PubMed: 19164570]
26. White CL, et al. Positioning cell wall synthetic complexes by the bacterial morphogenetic proteins MreB and MreD. *Molecular Microbiology*. 2010; 76:616–633. [PubMed: 20233306]
27. Ursell TS, et al. Rod-like bacterial shape is maintained by feedback between cell curvature and cytoskeletal localization. *Proc Natl Acad Sci USA*. 2014; doi: 10.1073/pnas.1317174111
28. Garner EC, et al. Coupled, circumferential motions of the cell wall synthesis machinery and MreB filaments in *B. subtilis*. *Science*. 2011; 333:222–225. [PubMed: 21636745]
29. Domínguez-Escobar J, et al. Processive movement of MreB-associated cell wall biosynthetic complexes in bacteria. *Science*. 2011; 333:225–228. [PubMed: 21636744]
30. van Teeffelen S, et al. The bacterial actin MreB rotates, and rotation depends on cell-wall assembly. *Proc Natl Acad Sci USA*. 2011; 108:15822–15827. [PubMed: 21903929]
31. Morgenstein RM, et al. RodZ links MreB to cell wall synthesis to mediate MreB rotation and robust morphogenesis. *Proc Natl Acad Sci USA*. 2015; 112:12510–12515. [PubMed: 26396257]
32. Ausmees N, et al. The bacterial cytoskeleton: an intermediate filament-like function in cell shape. *Cell*. 2003; 115:705–713. [PubMed: 14675535]
33. Cabeen MT, et al. Bacterial cell curvature through mechanical control of cell growth. *EMBO J*. 2009; 28:1208–1219. [PubMed: 19279668]
34. Cabeen MT, et al. Mutations in the Lipopolysaccharide biosynthesis pathway interfere with crescentin-mediated cell curvature in *Caulobacter crescentus*. *J Bacteriol*. 2010; 192:3368–3378. [PubMed: 20435724]
35. Charbon G, et al. Bacterial intermediate filaments: in vivo assembly, organization, and dynamics of crescentin. *Genes Dev*. 2009; 23:1131–1144. [PubMed: 19417107]
36. Ingerson-Mahar M, et al. The metabolic enzyme CTP synthase forms cytoskeletal filaments. *Nat Cell Biol*. 2010; 12:739–746. [PubMed: 20639870]
37. Persat A, et al. The curved shape of *Caulobacter crescentus* enhances surface colonization in flow. *Nat Commun*. 2014; 5:3824. [PubMed: 24806788]
38. Haeusser DP, Margolin W. Splitsville: structural and functional insights into the dynamic bacterial Z ring. *Nat Rev Microbiol*. 2016; 14:305–319. [PubMed: 27040757]
39. Din N, et al. Dominant C-terminal deletions of FtsZ that affect its ability to localize in *Caulobacter* and its interaction with FtsA. *Molecular Microbiology*. 1998; 27:1051–1063. [PubMed: 9535094]
40. Wang Y, et al. A set of *ftsZ* mutants blocked at different stages of cell division in *Caulobacter*. *Molecular Microbiology*. 2001; 40:347–360. [PubMed: 11309118]
41. Thanbichler M, Shapiro L. MipZ, a spatial regulator coordinating chromosome segregation with cell division in *Caulobacter*. *Cell*. 2006; 126:147–162. [PubMed: 16839883]
42. Li Z, et al. The structure of FtsZ filaments in vivo suggests a force-generating role in cell division. *EMBO J*. 2007; 26:4694–4708. [PubMed: 17948052]
43. Holden SJ, et al. High throughput 3D super-resolution microscopy reveals *Caulobacter crescentus* in vivo Z-ring organization. *Proc Natl Acad Sci USA*. 2014; 111:4566–4571. [PubMed: 24616530]
44. Kiekebusch D, et al. Localized Dimerization and Nucleoid Binding Drive Gradient Formation by the Bacterial Cell Division Inhibitor MipZ. *Mol Cell*. 2012; doi: 10.1016/j.molcel.2012.03.004
45. Möll A, Thanbichler M. FtsN-like proteins are conserved components of the cell division machinery in proteobacteria. *Molecular Microbiology*. 2009; 72:1037–1053. [PubMed: 19400794]
46. Möll A, et al. DipM, a new factor required for peptidoglycan remodelling during cell division in *Caulobacter crescentus*. *Molecular Microbiology*. 2010; 77:90–107. [PubMed: 20497502]

47. Poggio S, et al. A protein critical for cell constriction in the Gram-negative bacterium *Caulobacter crescentus* localizes at the division site through its peptidoglycan-binding LysM domains. *Molecular Microbiology*. 2010; 77:74–89. [PubMed: 20497503]
48. Costa T, et al. Localization of PBP3 in *Caulobacter crescentus* is highly dynamic and largely relies on its functional transpeptidase domain. *Molecular Microbiology*. 2008; 70:634–651. [PubMed: 18786147]
49. Martin ME, et al. Cell cycle-dependent abundance, stability and localization of FtsA and FtsQ in *Caulobacter crescentus*. *Molecular Microbiology*. 2004; 54:60–74. [PubMed: 15458405]
50. Goley ED, et al. Imaging-based identification of a critical regulator of FtsZ protofilament curvature in *Caulobacter*. *Mol Cell*. 2010; 39:975–987. [PubMed: 20864042]
51. Meier EL, et al. A novel membrane anchor for FtsZ is linked to cell wall hydrolysis in *Caulobacter crescentus*. *Molecular Microbiology*. 2016; 101:265–280. [PubMed: 27028265]
52. Meeske AJ, et al. SEDS proteins are a widespread family of bacterial cell wall polymerases. *Nature*. 2016; 537:634–638. [PubMed: 27525505]
53. Cho H, et al. Bacterial cell wall biogenesis is mediated by SEDS and PBP polymerase families functioning semi-autonomously. *Nat Microbiol*. 2016; doi: 10.1038/nmicrobiol.2016.172
54. Goley ED, et al. DipM links peptidoglycan remodelling to outer membrane organization in *Caulobacter*. *Molecular Microbiology*. 2010; 77:56–73. [PubMed: 20497504]
55. Modell JW, et al. A DNA damage checkpoint in *Caulobacter crescentus* inhibits cell division through a direct interaction with FtsW. *Genes Dev*. 2011; 25:1328–1343. [PubMed: 21685367]
56. Modell JW, et al. A DNA Damage-Induced, SOS-Independent Checkpoint Regulates Cell Division in *Caulobacter crescentus*. *PLoS Biol*. 2014; 12:e1001977. [PubMed: 25350732]
57. Sundararajan K, et al. The bacterial tubulin FtsZ requires its intrinsically disordered linker to direct robust cell wall construction. *Nat Commun*. 2015; 6:7281. [PubMed: 26099469]
58. Bisson Filho AW, et al. Treadmilling by FtsZ filaments drives peptidoglycan synthesis and bacterial cell division. *Science*. 2017; 355:739–743. [PubMed: 28209898]
59. Yang X, et al. GTPase activity-coupled treadmilling of the bacterial tubulin FtsZ organizes septal cell-wall synthesis. *Science*. 2017; 355:744–747. [PubMed: 28209899]
60. Osawa M, et al. Reconstitution of contractile FtsZ rings in liposomes. *Science*. 2008; 320:792–794. [PubMed: 18420899]
61. Erickson HP, et al. FtsZ in bacterial cytokinesis: cytoskeleton and force generator all in one. *Microbiol Mol Biol Rev*. 2010; 74:504–528. [PubMed: 21119015]
62. Xiao J, Goley ED. Redefining the roles of the FtsZ-ring in bacterial cytokinesis. *Curr Opin Microbiol*. 2016; 34:90–96. [PubMed: 27620716]
63. Wagner JK, et al. A nutrient uptake role for bacterial cell envelope extensions. *Proc Natl Acad Sci USA*. 2006; 103:11772–11777. [PubMed: 16861302]
64. Poindexter JS, Staley JT. *Caulobacter* and *Asticcacaulis* stalk bands as indicators of stalk age. *J Bacteriol*. 1996; 178:3939–3948. [PubMed: 8682801]
65. Schlimpert S, et al. General Protein Diffusion Barriers Create Compartments within Bacterial Cells. *Cell*. 2012; 151:1270–1282. [PubMed: 23201141]
66. Skerker JM, et al. Two-component signal transduction pathways regulating growth and cell cycle progression in a bacterium: a system-level analysis. *PLoS Biol*. 2005; 3:e334. [PubMed: 16176121]
67. Sommer JM, Newton A. Turning off flagellum rotation requires the pleiotropic gene *pleD*: *pleA*, *pleC*, and *pleD* define two morphogenic pathways in *Caulobacter crescentus*. *J Bacteriol*. 1989; 171:392–401. [PubMed: 2536661]
68. Biondi EG, et al. A phosphorelay system controls stalk biogenesis during cell cycle progression in *Caulobacter crescentus*. *Molecular Microbiology*. 2006; 59:386–401. [PubMed: 16390437]
69. Wagner JK, et al. *Caulobacter crescentus* requires RodA and MreB for stalk synthesis and prevention of ectopic pole formation. *J Bacteriol*. 2005; 187:544–553. [PubMed: 15629926]
70. Kühn J, et al. Bactofilins, a ubiquitous class of cytoskeletal proteins mediating polar localization of a cell wall synthase in *Caulobacter crescentus*. *EMBO J*. 2010; 29:327–339. [PubMed: 19959992]

71. Hughes HV, et al. Co-ordinate synthesis and protein localization in a bacterial organelle by the action of a penicillin-binding-protein. *Molecular Microbiology*. 2013; 90:1162–1177. [PubMed: 24118129]
72. Hughes HV, et al. Protein localization and dynamics within a bacterial organelle. *Proc Natl Acad Sci USA*. 2010; 107:5599–5604. [PubMed: 20212131]
73. Quardokus E, et al. Cell cycle regulation and cell type-specific localization of the FtsZ division initiation protein in *Caulobacter*. *Proc Natl Acad Sci USA*. 1996; 93:6314–6319. [PubMed: 8692812]
74. Laub MT, et al. Global analysis of the genetic network controlling a bacterial cell cycle. *Science*. 2000; 290:2144–2148. [PubMed: 11118148]
75. Kelly AJ, et al. Cell cycle-dependent transcriptional and proteolytic regulation of FtsZ in *Caulobacter*. *Genes Dev*. 1998; 12:880–893. [PubMed: 9512521]
76. Williams B, et al. ClpXP and ClpAP proteolytic activity on divisome substrates is differentially regulated following the *Caulobacter* asymmetric cell division. *Molecular Microbiology*. 2014; doi: 10.1111/mmi.12698
77. Schrader JM, et al. The Coding and Noncoding Architecture of the *Caulobacter crescentus* Genome. *PLoS Genet*. 2014; 10:e1004463. [PubMed: 25078267]
78. Zhou B, et al. The global regulatory architecture of transcription during the *Caulobacter* cell cycle. *PLoS Genet*. 2015; 11:e1004831. [PubMed: 25569173]
79. Radhakrishnan SK, et al. Coupling prokaryotic cell fate and division control with a bifunctional and oscillating oxidoreductase homolog. *Dev Cell*. 2010; 18:90–101. [PubMed: 20152180]
80. Joshi KK, et al. An Adaptor Hierarchy Regulates Proteolysis during a Bacterial Cell Cycle. *Cell*. 2015; 163:419–431. [PubMed: 26451486]
81. Janakiraman B, et al. In-phase oscillation of global regulons is orchestrated by a pole-specific organizer. *Proc Natl Acad Sci USA*. 2016; 113:12550–12555. [PubMed: 27791133]
82. Gonin M, et al. Regulation of stalk elongation by phosphate in *Caulobacter crescentus*. *J Bacteriol*. 2000; 182:337–347. [PubMed: 10629178]
83. Poindexter JS. The role of calcium in stalk development and in phosphate acquisition in *Caulobacter crescentus*. *Arch Microbiol*. 1984; 138:140–152. [PubMed: 6477030]
84. Klein EA, et al. Physiological role of stalk lengthening in *Caulobacter crescentus*. *Commun Integr Biol*. 2013; 6:e24561. [PubMed: 23986806]
85. Poindexter JS. Biological Properties and Classification of the *Caulobacter* Group. *Bacteriol Rev*. 1964; 28:231–295. [PubMed: 14220656]
86. Hoffman MD, et al. Timescales and Frequencies of Reversible and Irreversible Adhesion Events of Single Bacterial Cells. *Anal Chem*. 2015; 87:12032–12039. [PubMed: 26496389]
87. Jiang C, et al. Sequential evolution of bacterial morphology by co-option of a developmental regulator. *Nature*. 2014; 506:489–493. [PubMed: 24463524]
88. Lubin EA, et al. Identification of the PhoB Regulon and Role of PhoU in the Phosphate Starvation Response of *Caulobacter crescentus*. *J Bacteriol*. 2015; 198:187–200. [PubMed: 26483520]
89. Boutte CC, Crosson S. The complex logic of stringent response regulation in *Caulobacter crescentus*: starvation signalling in an oligotrophic environment. *Molecular Microbiology*. 2011; 80:695–714. [PubMed: 21338423]
90. Lesley JA, Shapiro L. SpoT regulates DnaA stability and initiation of DNA replication in carbon-starved *Caulobacter crescentus*. *J Bacteriol*. 2008; 190:6867–6880. [PubMed: 18723629]
91. Chiaverotti TA, et al. Conditions that trigger guanosine tetraphosphate accumulation in *Caulobacter crescentus*. *J Bacteriol*. 1981; 145:1463–1465. [PubMed: 7204347]
92. Gonzalez D, Collier J. Effects of (p)ppGpp on the progression of the cell cycle of *Caulobacter crescentus*. *J Bacteriol*. 2014; 196:2514–2525. [PubMed: 24794566]
93. Stott KV, et al. (p)ppGpp modulates cell size and the initiation of DNA replication in *Caulobacter crescentus* in response to a block in lipid biosynthesis. *Microbiology*. 2015; 161:553–564. [PubMed: 25573769]
94. Hugonnet J-E, et al. Factors essential for L,D-transpeptidase-mediated peptidoglycan cross-linking and β -lactam resistance in *Escherichia coli*. *eLife*. 2016:5.

95. Steinchen W, Bange G. The magic dance of the alarmones (p)ppGpp. *Molecular Microbiology*. 2016; 101:531–544. [PubMed: 27149325]
96. Sanselicio S, Viollier PH. Convergence of Alarmone and Cell Cycle Signaling from Trans-Encoded Sensory Domains. *mBio*. 2015; 6:e01415–15. [PubMed: 26489861]
97. Leslie DJ, et al. Nutritional Control of DNA Replication Initiation through the Proteolysis and Regulated Translation of DnaA. *PLoS Genet*. 2015; 11:e1005342. [PubMed: 26134530]
98. Balhasteros H, et al. CspC and CspD are essential for *Caulobacter crescentus* stationary phase survival. *Arch Microbiol*. 2010; 192:747–758. [PubMed: 20607520]
99. da Silva CAPT, et al. Transcriptomic analysis of the stationary phase response regulator SpdR in *Caulobacter crescentus*. *BMC Microbiol*. 2016; 16:66. [PubMed: 27072651]
100. da Silva CAPT, et al. SpdR, a response regulator required for stationary-phase induction of *Caulobacter crescentus cspD*. *J Bacteriol*. 2010; 192:5991–6000. [PubMed: 20833806]
101. Campos M, et al. A Constant Size Extension Drives Bacterial Cell Size Homeostasis. *Cell*. 2014; 159:1433–1446. [PubMed: 25480302]
102. Banerjee S, et al. Crossover in the dynamics of cell wall growth controls *Caulobacter* division times. *bioRxiv*. 2016; doi: 10.1101/047589
103. Harris LK, et al. A *Caulobacter* MreB mutant with irregular cell shape exhibits compensatory widening to maintain a preferred surface area to volume ratio. *Molecular Microbiology*. 2014; 94:988–1005.
104. Harris LK, Theriot JA. Relative Rates of Surface and Volume Synthesis Set Bacterial Cell Size. *Cell*. 2016; 165:1479–1492. [PubMed: 27259152]
105. Hocking J, et al. Osmolality-Dependent Relocation of Penicillin-Binding Protein PBP2 to the Division Site in *Caulobacter crescentus*. *J Bacteriol*. 2012; 194:3116–3127. [PubMed: 22505677]
106. Koyasu S, et al. Penicillin-binding Proteins of the Stalk of *Caulobacter crescentus*. *Microbiology*. 1983; 129:2789–2799.
107. Cabeen MT, et al. The domain organization of the bacterial intermediate filament-like protein crescentin is important for assembly and function. *Cytoskeleton (Hoboken)*. 2011; 68:205–219. [PubMed: 21360832]
108. Wang L, Lutkenhaus J. FtsK is an essential cell division protein that is localized to the septum and induced as part of the SOS response. *Molecular Microbiology*. 1998; 29:731–740. [PubMed: 9723913]
109. Yeh YC, et al. The *Caulobacter* Tol-Pal complex is essential for outer membrane integrity and the positioning of a polar localization factor. *J Bacteriol*. 2010; 192:4847–4858. [PubMed: 20693330]

Trends

- *Caulobacter* cell shape arises through spatiotemporal regulation of cell wall synthesis.
- Cell width, length, curvature, and stalk formation are the major shape attributes for *Caulobacter* cells, and are regulated by the MreB, FtsZ, crescentin, and bactofilin cytoskeletons, respectively. Though independently regulated, these shape features are coordinated in time and space.
- *Caulobacter* length and stalk formation are cell-cycle regulated through transcriptional, translational and proteolytic control, as well as through post-translational modifications and spatial regulation.
- Starvation, stationary phase, and other stresses promote distinct morphological adaptations to facilitate survival. The mechanisms of shape adaptation are mostly unclear, but include transcriptional responses to environmental signals.
- Quantitative principles describe shape homeostasis in *Caulobacter*.

Figure 1.

Caulobacter cell shape is dynamically regulated through cytoskeletal control of cell wall synthesis. **(A)** Morphological changes that occur as *Caulobacter* progresses through the cell cycle. Changes in the localization of the cytoskeletal proteins, FtsZ, MreB, crescentin and bactofilins are depicted. **(B)** Consequences of inactivation individual cytoskeletal proteins on cell morphology. *cresS* encodes crescentin. **(C)** Depiction of the *Caulobacter* stalk with all of the layers of the cell envelope and localization of stalk proteins StpX and PbpC. Abbreviations: IM, inner membrane; PG, peptidoglycan; OM, outer membrane; SB, stalk cross band.

Figure 2.

Environmental changes influence *Caulobacter* cell morphology. **(1)** Under normal, nutrient-replete conditions, swarmer cells differentiate to become stalked cells (black arrow). **(2)** (p)ppGpp production upon induction of the stringent response slows, but does not completely inhibit, the swarmer-to-stalked transition (dashed red line) and **(3)** causes cells to become smaller. **(4)** Phosphate starvation causes *Caulobacter* cells to elongate their stalks and to increase in cell volume. **(5)** In late stationary phase, cells become filamentous and adopt a coiled or helical morphology.

Table 1

Factors important for specification and maintenance of *Caulobacter* cell width, curvature, length, and stalk formation.

Shape Feature	Protein	Role	Essential?	Loss-of-Function Phenotype	Refs
Width					
	MreB	Cytoskeletal protein, likely senses local envelope curvature, acts as scaffold, directs PG metabolism	Yes	Cell rounding, lemon shape, rounded stalks	[13–16, 21, 27, 69]
	RodZ	Required for circumferential movement of MreB	Yes	Cell rounding, lemon shape	[25, 26, 31]
	RodA	Likely PG glycosyltransferase	Yes	Cell rounding, lemon shape, rounded stalks	[24, 69]
	MblA	Binds MreB, regulates MreB localization	No	None, but cell rounding when overexpressed	[20]
	PBP2	PG transpeptidase	Yes	Increased cell width, rounded stalks	[22, 23, 105, 106]
	MreC	Unclear	Yes	Cell rounding, lemon shape	[22–24]
	MreD	Unclear	Yes	Cell rounding, lemon shape	[24, 26]
Curvature					
	CreS/crescentin	Cytoskeletal protein, mechanically regulates PG remodeling	No	Straight cells	[32, 33, 35, 107]
	CtpS	CTP synthase, negatively regulates cell curvature	Yes	Hypercurved cells	[36]
	MreB	Mediates crescentin attachment to membrane?	Yes	Cell rounding on depletion or inhibition; some viable point mutants have aberrant curvature and disrupted crescentin localization	[21, 35]
Length					
	FtsZ	Cytoskeletal protein, acts as scaffold, directs PG metabolism, generates force?	Yes	Filamentation, no constrictions	[17, 18, 40]
	MipZ	Coordinates division with chromosome segregation in time and space	No, but cells very sick without it	Filamentation, heterogeneous cell length	[41, 79]
	FtsA	Membrane anchor for FtsZ	Yes	Filamentation, some shallow constrictions	[49]
	FtsQ	Promotes divisome stability	Yes	Filamentation, some shallow constrictions	[49]
	FzIA	FtsZ binding protein	Yes	Filamentation, no constrictions	[50]
	ZapA	FtsZ binding protein	No	Cell elongation, heterogeneous cell length	[17]
	FtsE	ABC transporter family, ATPase subunit (with FtsX)	No	Cell elongation, cell chaining	[17]
	FtsX	ABC transporter family, transmembrane subunit (with FtsE)	Yes	Unknown	[17]
	FtsK	DNA translocase, promotes divisome stability	Yes	Cell filamentation, shallow constrictions, missegregation of chromosomal termini	[108]

Shape Feature	Protein	Role	Essential?	Loss-of-Function Phenotype	Refs
	FtsL	Promotes divisome stability, protein recruitment	Yes	Cell filamentation, no/few constrictions	[17]
	FtsB	Promotes divisome stability	No	None apparent	[17]
	FtsN	PG binding protein, promotes divisome stability	Yes	Cell filamentation, no constrictions	[45]
	FtsI	PG transpeptidase	Yes	Cell filamentation, no constrictions	[48]
	FtsW	Likely PG glycosyltransferase	Yes	Cell filamentation, no constrictions	[17]
	DipM	Regulates PG hydrolysis	No	Cell chaining, thickened cell wall, thickened septa, outer membrane blebbing	[46, 47, 54]
	Tol-Pal complex	Maintains envelope geometry	Yes	Outer membrane blebbing, particularly at division site	[109]
Stalk					
	BacA	Cytoskeletal protein, recruits PbpC	No	Stalks do not elongate properly during phosphate starvation, PbpC does not localize	[70]
	BacB	Cytoskeletal protein, recruits PbpC	No	Stalks do not elongate properly during phosphate starvation	[70]
	PbpC	Bifunctional PBP	No	Stalks do not elongate properly during phosphate starvation, SipX does not localize	[70, 71]
	SipX	Role in metal homeostasis?	No	Stalks do not elongate properly during phosphate starvation	[71, 72]
	SipABCD	Make up stalk cross-bands	No	Not as fit as wild type in competition assay, diffusion barrier between stalk and cell body is lost	[65]