CellPress

Primer

Caulobacter crescentus: model system extraordinaire

Sander K. Govers[1,2] and Christine Jacobs-Wagner[1,2,*]

In scientific research, we often rely on well-established model systems to tackle important questions. In this context, extensive characterization of specific bacterial species such as *Escherichia coli* and *Bacillus subtilis* has provided a vast amount of knowledge that extends well beyond the biology of these two organisms. However, the bacterial world is large and extremely diverse, necessitating the development of additional models that complement the classical rod-shaped and symmetrically dividing systems. *Caulobacter crescentus* is a species that has met this need effectively, as its dimorphic lifestyle showcases distinctive features, including cellular asymmetry and differentiation during the cell cycle. Studying *C. crescentus* has reformed our understanding of bacterial intracellular organization, cellular development, and cell-cycle regulation. These findings have, in turn, stimulated studies in other bacteria, shedding light on how protein function and cell morphology can evolve and diversify. Studies in *C. crescentus* have also deepened our knowledge of other topics (e.g. cell mechanosensing, motility, and bacterial aging), while opening the door to biotechnological innovations. In this Primer, we provide some general background to this peculiar bacterium and highlight specific features that have contributed to its rise as a versatile bacterial model. This Primer is not meant to be exhaustive on any topic and is instead intended to provide a taste of the power of *C. crescentus* as a model system to explore a diverse range of topics.

A freshwater bacterium with unusual morphogenetic features and a dimorphic life cycle

Caulobacter crescentus belongs to the order of the Caulobacterales, a group of bacteria characterized by the presence of a stalk, which is a thin extension of the cell body (Figure 1A). Arthur Henrici and Delia Johnson first described this bacterial order in 1935 after capturing several stalked bacterial species by submerging microscope slides in a freshwater lake. Caulobacterales simply stuck to the slides via a polysaccharide adhesive (holdfast) present at the tip of their stalks (Figure 1A). The most famous member of this order, *Caulobacter crescentus*, named after its characteristic crescent shape (Figure 1A), was described by Jeanne Poindexter in 1964 after isolating the species from pond water in California.

Starting in the 1970s, pioneering efforts primarily by Bert Ely, Austin Newton, Lucy Shapiro and their coworkers provided key molecular insights into the life cycle of this organism, established genetic tools and uncovered the biochemical and regulatory function of specific proteins. This was followed by seminal papers on the cell-cycle regulation and cell biology of *C. crescentus* from the laboratory of Lucy Shapiro, whose work inspired a generation of researchers. Since then,

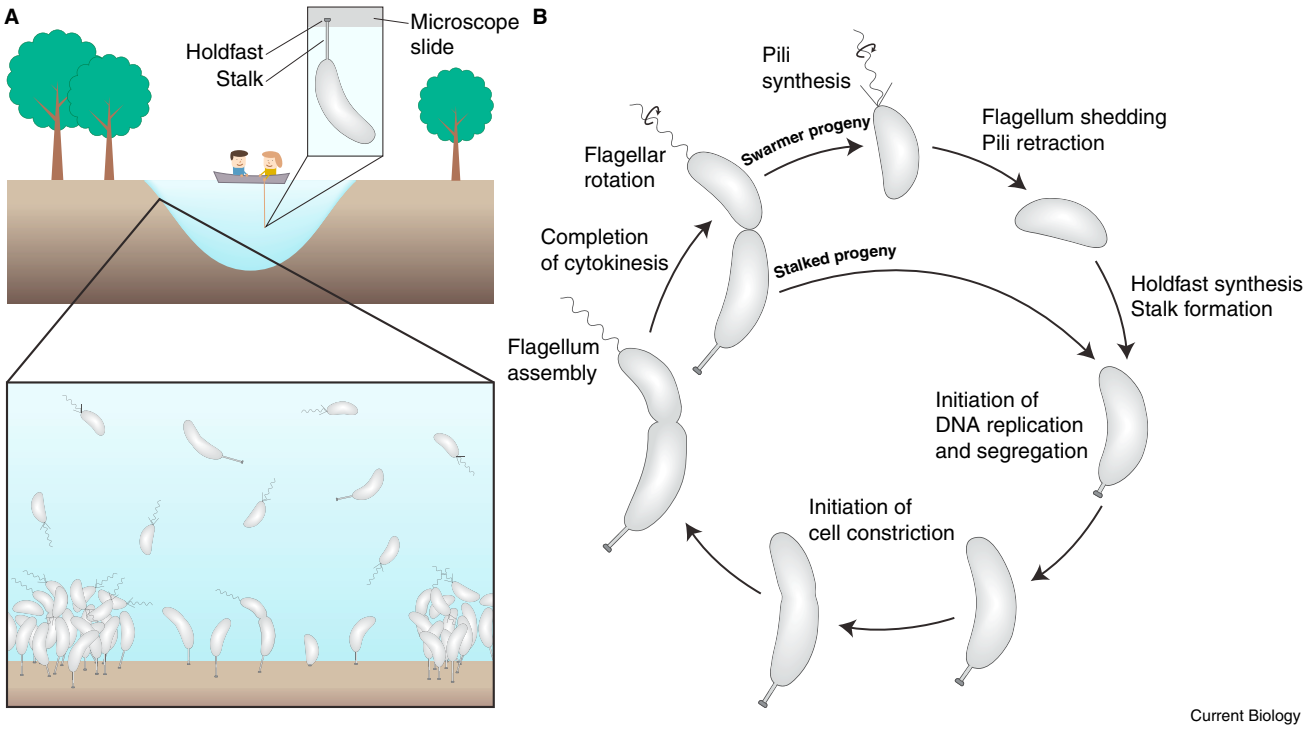

Figure 1. *C. crescentus* ecology and life cycle.
(A) Schematic showing *C. crescentus* cells in their natural habitat, fresh water, in which they live planktonically or in biofilms, through holdfast-mediated surface attachment. (B) Schematic of the *C. crescentus* dimorphic life cycle. Developmental and cell-cycle events are indicated at each step.

contributions from many laboratories across the world have transformed this innocuous bacterium into one of today's valued model systems for uncovering the mysteries of the bacterial world.

But first, let us start with what initially made *C. crescentus* special. This organism thrives in nutrient-poor aquatic environments (e.g. lakes and streams) through various cellular adaptations. For example, the stalk, which can elongate under starvation conditions, expands the surface area of the cell envelope and enhances nutrient uptake by increasing the surface-to-volume ratio. In addition, synthesis of the holdfast promotes surface attachment, adhesion to other cells and biofilm formation (Figure 1A). Numerous benefits are associated with a biofilm lifestyle, including protection against predation and environmental stresses. Nevertheless, a balance between attachment and planktonic dispersal remains crucial, especially when the local environment deteriorates. *C. crescentus* achieves this balance through its remarkable dimorphic life cycle, which it shares with other members of the *Caulobacter* genus. In contrast to *E. coli* and *B. subtilis*, *C. crescentus* divides asymmetrically (Figure 1B), producing genetically identical but morphologically distinct daughter cells: a sessile stalked cell and a smaller, flagellated swarmer cell (Figure 1B). Following cell division, the stalked progeny initiates DNA replication and continues cell-cycle progression until the subsequent asymmetric cell division. The swarmer progeny first goes through a motile period in the absence of DNA replication, after which it sheds the flagellum, retracts the pili and differentiates into a stalked cell. The dimorphic life cycle ensures persistence of both cell types and is believed to serve as a bet-hedging strategy. The dispersal of swarmer cells allows the exploration of new niches while minimizing competition for resources with sessile stalked cells. Stalked cells, on the other hand, ensure cellular proliferation and generation of new swarmer cells.

A highly regulated dimorphic life cycle

The dimorphic life cycle of *C. crescentus* requires tight regulation to ensure faithful coordination between developmental programs and cell-cycle events. *C. crescentus* is uniquely suited for studying cell development and cell-cycle regulation due to the ease of isolating swarmer cells from a *C. crescentus* population via density gradient centrifugation. This is possible because swarmer cells lack a polysaccharide capsule and are therefore less buoyant than the encapsulated cells at later cell-cycle stages. After resuspension into growth medium, the isolated swarmer cells resume cell-cycle progression in a synchronized fashion, allowing for biochemical and genomic characterization at specific stages of the cell cycle. This synchronization method has transformed *C. crescentus* into a powerful model bacterium for investigating the signaling and regulatory networks underlying cell-cycle progression, cellular differentiation and asymmetric division. Genome-wide analyses of such synchronized cultures have revealed that large fractions of the *C. crescentus* transcriptome (~10–40%), proteome (~15%) and metabolome (~14%) vary as a function of the cell cycle, providing evidence of extensive cell-cycle coordination. Such a high level of cell-cycle dependency is reminiscent of that observed in eukaryotes. The eukaryotic cell cycle is a regulated, ordered and directional process, characterized by checkpoints, cell-cycle transcriptional regulators, cyclins, cyclin-dependent kinases, intricate signaling networks, multiple levels of regulation and metabolic oscillations. Such features were thought to be unique to eukaryotic cell cycles, but *C. crescentus* research shattered this notion, as illustrated below.

Early genetic observations already revealed that *C. crescentus* has several checkpoints in place to ensure that its developmental programs are properly coordinated with cell-cycle progression (Figure 1B). For example, flagellum synthesis requires the initiation of DNA replication, while the activation of flagellar rotation and the synthesis of pili depend on the completion of distinct steps of cell division.

It was later shown that the *C. crescentus* cell cycle also showcases master regulators. Among them, DnaA, CtrA and GcrA directly affect the expression of numerous genes by acting as transcriptional regulators, whereas a fourth master regulator, CcrM, regulates transcription through DNA methylation at specific sites along the genome. Each master regulator also affects the expression of another master regulator, resulting in out-of-phase oscillations such that each is most abundant and active during a discrete period of the cell cycle (Figure 2A). This self-sustained oscillatory behavior leads to a temporally regulated sequence of gene expression across the cell cycle.

Cell-cycle regulation does not act solely at the transcriptional level. This is best exemplified with CtrA, an essential response regulator that is involved in the regulation of cell division, flagellum synthesis, holdfast production, pili generation, chromosome methylation and DNA replication initiation. CtrA activity is controlled at multiple levels, including not only transcription, but also protein degradation and phosphorylation through a complex network of regulatory proteins (Figure 2B). Activation of CtrA by phosphorylation occurs through a phosphorelay system that consists of CckA, a hybrid histidine kinase/phosphatase, and ChpT, a phosphotransferase that shuttles phosphate groups between CckA and CtrA (Figure 2B). This phosphorelay also controls the phosphorylation of the CpdR response regulator, which, when unphosphorylated, functions as an adaptor protein that stimulates CtrA proteolysis (Figure 2B). Through phosphorylation or dephosphorylation, CckA thus controls both the activity and stability of CtrA. The switch between CckA's opposing activities is controlled by multiple, interconnected two-component signal transduction proteins — PleC/PleD, DivJ/DivK and DivL (Figure 2B). In addition, CckA activity is controlled by the second messenger cyclic diguanylate (c-di-GMP), which directly stimulates CckA's phosphatase activity (Figure 2B). The concentration of c-di-GMP is low in swarmer cells but increases and peaks during the swarmer-to-stalked cell transition, which leads to the inactivation and degradation of CtrA, followed by the onset of DNA replication. To the best of our knowledge, these cell-cycle-dependent oscillations of c-di-GMP represented the first evidence of a metabolite changing in abundance during a bacterial cell cycle.

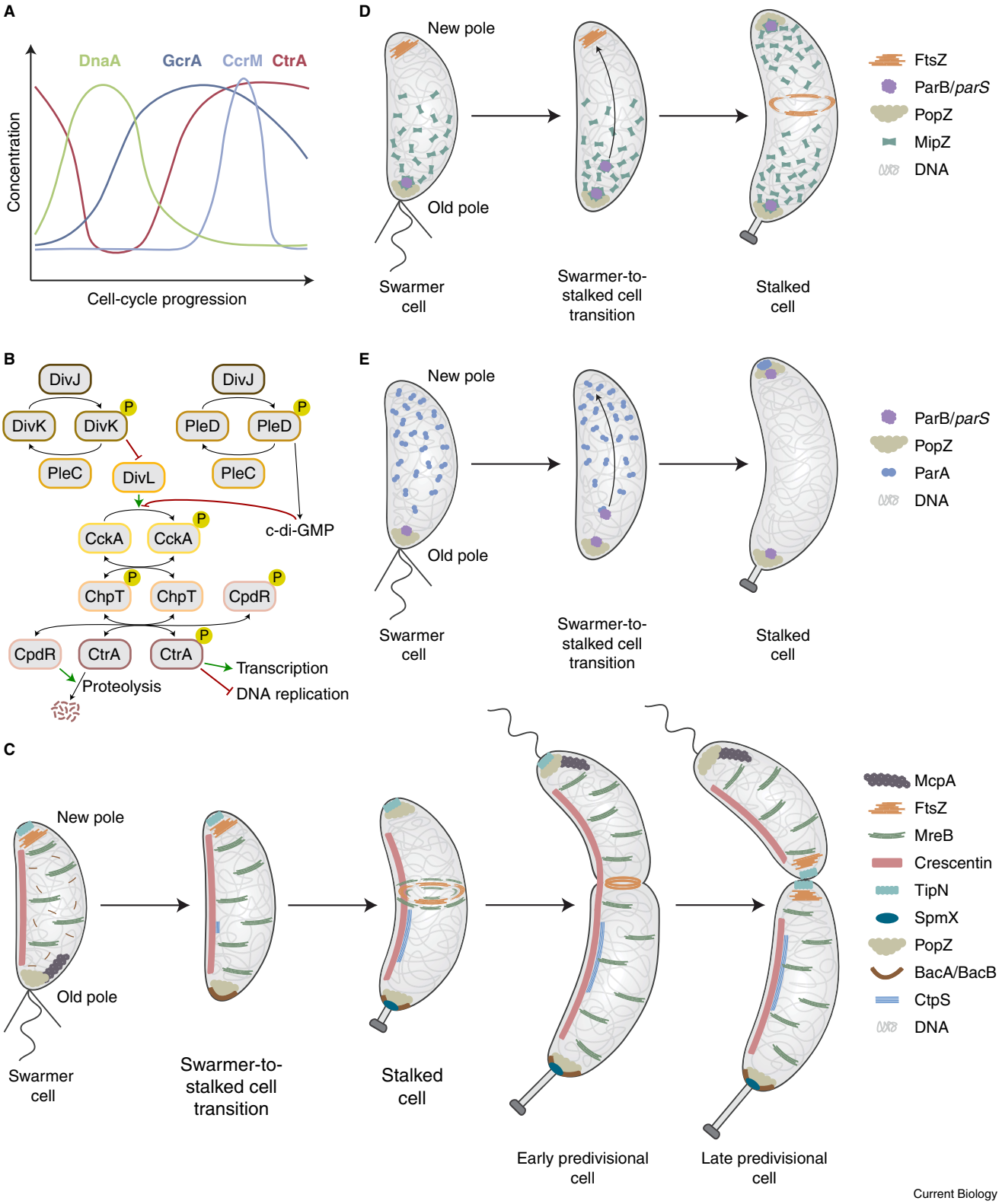

Figure 2. Examples of intracellular organization and cell-cycle regulation in *C. crescentus*.
(A) Out-of-phase concentration oscillations of master regulators across the *C. crescentus* cell cycle. (B) Schematic of the regulatory network that controls CtrA activity. (C) Dynamic subcellular localization of indicated proteins during the *C. crescentus* cell cycle. (D) Establishment of the MipZ protein gradient for FtsZ ring positioning in stalked cells. (E) Translocation of the ParB–*parS* partition complex across the ParA gradient during chromosome segregation.

The *C. crescentus* cell cycle thus exhibits extensive control mechanisms, integrating multiple input signals by using a combination of oscillating master regulators, phosphosignaling networks, metabolic oscillations and checkpoint controls. This degree of regulation illustrates how a cell can achieve the amazing feat of coordinating complex morphogenic and cell-cycle processes in both time and space.

An intricate spatial and temporal intracellular organization

The dimorphic lifestyle of *C. crescentus* also requires differentiation of both cell poles prior to cell division. Cell polarity is morphologically apparent by the presence of polar appendages (flagellum, pili, stalk) and the asymmetric division that generates progeny of unequal size. Establishment of cell polarity occurs intracellularly by the precise spatiotemporal patterning of key effectors and regulatory proteins that integrate cell morphogenesis and development with cell-cycle progression. These properties have made *C. crescentus* an attractive bacterial model system for understanding complex intracellular organizational schemes that were once thought to be exclusively eukaryotic. As a result, *C. crescentus* has helped birth the ever-expanding field of bacterial cell biology.

It probably all started in 1992 with striking immunogold-stained electron micrographs of *C. crescentus* cells showing that the chemoreceptor McpA is always found near the flagellated cell pole (Figure 2C). The polar localization of chemoreceptors is now well known to be common across bacterial species, including those with randomly distributed flagella. Early implementation of fluorescence microscopy techniques led to the identification of many more proteins with distinct localization patterns in *C. crescentus*. In fact, a considerable fraction (~10%) of the *C. crescentus* proteome displays a specific, and often dynamic, subcellular organization. Subcellular localization is not limited to proteins related to the polar organelles; it is also exhibited by some signal transduction proteins, transcriptional regulators, enzymes, protease components, and scaffold proteins.

In addition, *C. crescentus* has a considerable repertoire of filament-forming proteins that are often found at specific subcellular locations. In addition to the well-known tubulin and actin homologs, FtsZ and MreB (Figure 2C), *C. crescentus* possesses an intermediate-filament-like protein — crescentin — the first to be discovered in bacteria. Crescentin generates the distinctive curved morphology of *C. crescentus* by self-assembling into a filamentous structure along one side of the cell and bending the cell through anisotropic cell wall growth (Figure 2C). Intermediate-filament-like proteins have since been discovered in other bacterial species such as *Streptomyces coelicolor* and *Helicobacter pylori*, where they organize polar growth and contribute to cell shape, respectively. Another class of bacterial cytoskeletal proteins first discovered in *C. crescentus* are bactofilins. These polymer-forming proteins are widely conserved among bacteria and unique to this domain of life. Bactofilin function differs between species and includes roles in cell morphogenesis, motility, cell division and chromosome organization. In *C. crescentus*, bactofilins BacA and BacB function as scaffolds for a cell-wall enzyme involved in stalk biogenesis. Their localization is cell-cycle regulated and transitions from a diffuse pattern in swarmer cells to an accumulation at the old cell pole during the swarmer-to-stalked cell transition (Figure 2C). Another fascinating cell biological discovery in *C. crescentus* was the realization that the CTP synthase, CtpS, is capable of polymerizing (Figure 2C), a property conserved in yeast, flies and mammals. Polymerization likely serves a regulatory function, as it impedes CtpS activity and is required for feedback inhibition.

C. crescentus has also taught us that bacteria, like eukaryotes, use polarity factors to help establish cell polarity. Polarity factors serve as spatial cues that orient the polarity axis of the cell. One example in *C. crescentus* is TipN, a transmembrane protein that marks the new cell pole by tracking where the last division occurred (Figure 2C). This birthmark ensures that a new flagellum will be synthesized at the new pole of predivisional cells and not elsewhere. Another transmembrane protein, SpmX, serves as a marker at the opposite cell pole, the old pole, where it promotes stalk biogenesis (Figure 2C). The recruitment of SpmX at the old pole is facilitated by PopZ, an intrinsically disordered, multivalent, pole-organizing factor that self-assembles into phase-separated microdomains. Microdomains are a means of subcellular organization that provide a secluded environment for a specific set of proteins. PopZ forms microdomains at the cell pole(s) by assembling into a porous matrix that selectively recruits and sequesters certain proteins. PopZ's transition from unipolar to bipolar localization during the swarmer-to-stalked cell transition (Figure 2C) is important for attaching duplicated chromosomes at opposite poles and coordinating regulatory programs with this cell-cycle event.

For some proteins, the situation is more complex than localizing to a specific subcellular site. As is the case for eukaryotic cells, bacterial cells can display protein gradients. This is quite surprising as the small size of bacterial cells and the fast diffusion of cytoplasmic proteins was expected to preclude the formation of such gradients. Bacteria have solved this problem by evolving simple self-organizing systems that combine a localized sink or source with a means of slowing protein diffusion. The Min system, which contributes to division site selection in *E. coli*, is a well-known example of a self-organizing system. In this case, protein diffusion is slowed through transient binding of the Min proteins to the membrane. However, the Min system is only found in a subset of bacteria, raising the question of how other species position their division machinery. In *C. crescentus*, this task is accomplished by bipolar gradients of MipZ, an inhibitor of FtsZ polymerization. The MipZ gradients lead to preferential assembly of the FtsZ cytokinetic ring near midcell where the concentration of MipZ is lowest (Figure 2D). The bipolar gradients are formed by interaction of MipZ with the DNA partitioning protein ParB at opposite cell poles (localized MipZ sources), leading to MipZ dimerization and subsequent association with the chromosomal DNA, thus slowing MipZ diffusion. MipZ therefore showcases how a protein gradient can be established in bacterial cells by using the chromosomal DNA (instead of the membrane) as a scaffold for reducing the rate of protein diffusion. The

same is true for the ParA gradient in *C. crescentus* swarmer cells (Figure 2E), except that, in this case, ParB, which is concentrated at the old cell pole, functions as a localized sink. ParB stimulates the dissociation of ParA dimers into monomers, leading to their release from the chromosomal DNA. ParA and ParB, together with the origin-proximal *parS* DNA sequence, form the *C. crescentus* ParABS system, a widely conserved active transport system for separating chromosomes and partitioning plasmids in bacteria. In *C. crescentus*, chromosome segregation is initiated by the interaction of a ParB–*parS* partition complex with the DNA-bound ParA gradient (Figure 2E). Remarkably, the active transport of this partition complex does not involve cytoskeletal filaments or motors. Instead, evidence suggests a new form of active transport in which the ParA dimers serve as transient tethers that harness the intrinsic elastic dynamics of the chromosome to translocate the partition complex across the cell.

The aforementioned examples demonstrate how intracellular protein gradients can arise in tiny cells, using a limited number of molecular components. They also illustrate the role of such gradients in different essential cellular processes, including division-site positioning and chromosome segregation and organization. Additional insight into the higher-order chromosome organization of *C. crescentus* came from single-cell microscopy and genome-wide chromosome conformation capture (Hi-C) experiments. The Hi-C experiments in *C. crescentus*, which were the first ones to be carried out in bacteria, mapped the domain organization of the chromosome at an unprecedented level of resolution and identified high transcriptional activity as a major determinant of domain formation.

A gateway to α-proteobacterial diversity

The discovery of intricate regulatory networks and subcellular organization in *C. crescentus* illustrates how a cell can integrate spatiotemporal information to coordinate cell-cycle events and cell morphogenesis. *C. crescentus* also serves as an example of how complex regulation can arise from connections between simple modules and how this

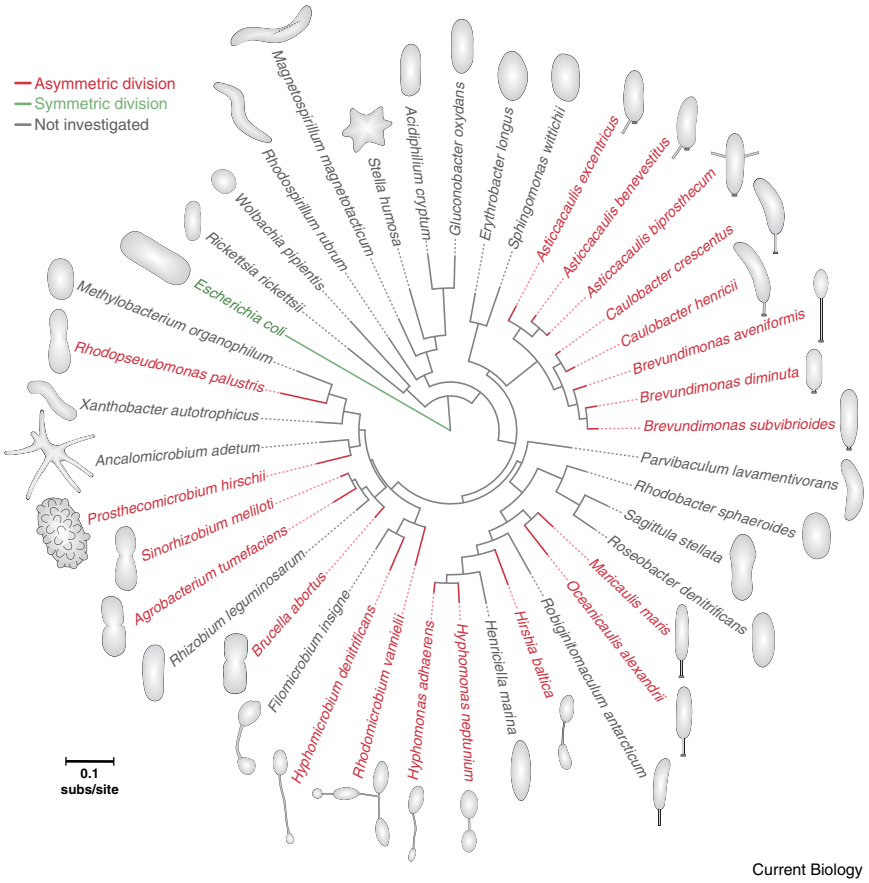

Figure 3. α-proteobacterial phylogeny and morphological diversity.
Phylogeny of selected species was derived from 16S rRNA sequences (using *E. coli* as an outgroup). The sequences were obtained from the Ribosomal Database Project and aligned using MUSCLE. The maximum likelihood tree was then generated using raxmlGUI 2.0 and the resulting tree was formatted using iTOL. Coloring of tree branches and species names indicates the type of cell division for a given species: red for species in which asymmetric cell division has been demonstrated, green for symmetrically dividing species, and grey for species in which cell division has not been carefully examined. Cell shapes are not drawn to scale.

complex regulation, through differential localization of regulatory proteins, can generate distinct cell fates among genetically identical daughter cells. This knowledge has motivated the search for, and examination of, similar regulatory networks and spatial organizations in other bacteria, especially in related species within the α-proteobacteria.

The α-proteobacteria are a fascinating class of bacteria with diversity in cellular morphology and lifestyle (Figure 3). Among its members are plant symbionts and pathogens (*Rhizobium*, *Agrobacterium*), arthropod endosymbionts (*Wolbachia*), intracellular pathogens (*Rickettsia*, *Brucella*), photosynthetic bacteria (*Rhodobacter*), bacteria that metabolize C1 compounds (*Methylobacterium*), magnetotactic bacteria (*Magnetospirillum*) and many prominent freshwater and marine genera (*Caulobacter*, *Maricaulis*, *Oceanicaulis*, *Roseobacter*) (Figure 3). Many components of the *C. crescentus* regulatory networks are conserved across α-proteobacteria. Detailed examination of these protein homologs has led to the discovery of cellular asymmetry in other species. For example, fluorescent labeling of a DivK homolog revealed that *Sinorhizobium meliloti* also divides asymmetrically, producing daughter cells with a distinct pattern of DivK localization. This led to the realization that *S. meliloti*, which was previously thought to divide symmetrically, also produces daughter cells of unequal size. Asymmetric division has since been demonstrated in many

other genera (*Brucella*, *Agrobacterium*, *Hyphomonas*, *Asticaccacaulis*, *Hirschia*, *Brevundimonas*, *Rhodopseudomonas*, *Prosthecomicrobium*, *Hyphomicrobium* and *Rhodomicrobium*), indicating that it may be prevalent throughout the α-proteobacteria (Figure 3).

Direct comparison of regulatory networks across species has also revealed striking plasticity and diversity in the exact function and regulation of their components. For example, the entire CckA–ChpT–CtrA/CpdR phosphorelay is conserved in the animal pathogen *Brucella abortus*, where it regulates cell division and the initiation of DNA replication, as in *C. crescentus*. Interestingly, though, the same phosphorelay is also required for intracellular survival in host cells. This is in line with the presence of CtrA-binding sites upstream of several *B. abortus* genes encoding virulence factors, indicating diversification of the CtrA regulon between the two species. In another example, DivJ, another component of the CtrA regulatory network, is essential for viability in *S. meliloti*, but not in *C. crescentus*, and is completely absent in some α-proteobacteria. This diversity and apparent modularity across species can be exploited to investigate why (ecologically) and how (mechanistically) developmental and morphological phenotypes have diversified across species (Figure 3).

An interesting case in this regard is that of stalk formation and positioning within the order of the Caulobacterales. Phylogenetic analysis indicates that an ancestral polar stalk, which is still present in *C. crescentus*, has been repositioned, increased in number or even lost in closely related genera (Figure 3). In the *Asticcacaulis* genus, stalk diversification (subpolar in *A. excentricus* and bilateral in *A. biprosthecum*) occurs through co-option of SpmX, the *C. crescentus* polar developmental regulator, as a stalk-positioning factor. An expansion of a specific region of the protein allowed for the acquisition of this new function and localization. Together, these findings indicate potential mechanisms for the evolution of bacterial morphology that likely extend beyond α-proteobacteria: evolution of protein function, co-option and modularity.

Cellular features with remarkable biophysical properties

The popularity *of C. crescentus* as a bacterial model system has expanded well beyond the study of cell development, cell polarity and morphological diversity. One example is motility. A wealth of knowledge on bacterial motility stems from *E. coli* and *Salmonella*, rod-shaped bacteria with multiple flagella randomly distributed along the cell body. However, many bacteria, including *C. crescentus*, have only a single flagellum at a specific location. Studying motility of the *C. crescentus* swarmer cell has therefore enriched our understanding of different types of swimming behavior. The *C. crescentus* flagellum is a right-handed helical filament that pushes the cell forward or pulls the cell backward depending on whether it is rotating clockwise or counterclockwise (Figure 4A). Its low torque (~350 pN nm versus ~1250 pN nm in the case of *E. coli*) provides swarmer cells with high swimming efficiency; the ratio of swimming speed to energy consumption rate of the flagellar motor is nearly 10-fold greater in *C. crescentus* than in *E. coli*. Motility is enhanced by the orientation of the cell body, which is tilted with respect to its swimming direction. This leads to a helical motion of the cell body that generates thrust and increases swimming efficiency (Figure 4A). The crescentin-induced curved shape of *C. crescentus* cells further increases the swimming efficiency and facilitates surface colonization in environments where cells are exposed to fluid flow. Both the high swimming efficiency and enhanced surface colonization ability are believed to be adaptations to aquatic and nutrient-poor environments. *C. crescentus* thus serves as an excellent example of how the interplay between cell shape and flagellar motor properties can be adapted to a species' surroundings.

Besides driving flagellar rotation, the flagellar motor has been implicated in the mechanosensing of surfaces (Figure 4B). Surface-induced motor interference leads to an increase in c-di-GMP production, which triggers the rapid synthesis of the holdfast to mediate permanent surface adhesion (Figure 4B). Another surface-sensing mechanism in *C. crescentus* involved in surface attachment is dependent on its tight-adherence (tad) pili (Figure 4B). Physical obstruction of pilus retraction also stimulates holdfast synthesis through c-di-GMP production, suggesting that resistance to pilus retraction is used for surface sensing. In addition, recent evidence suggests that obstruction of tad pili retraction not only stimulates holdfast synthesis, but also promotes initiation of DNA replication and cell differentiation in *C. crescentus* (Figure 4B). While mechanosensing is well established in eukaryotes, it remains a relatively new field in bacteria, pioneered by demonstrations of *E. coli* and *Pseudomonas aeruginosa* sensing surfaces using their flagella and pili. *C. crescentus* has proven to be a useful complementary model system to tackle the question of how bacteria respond to physical stimuli from their environment.

A door to technological applications and innovations

Holdfast synthesis in *C. crescentus* has also garnered attention for reasons outside of its direct link to the cell cycle. For one, the holdfast is a polysaccharide-based adhesin that is among the strongest biological adhesives characterized to date. In addition, the holdfast is gelatinous, elastic, biodegradable and able to adhere to a broad range of substrates, making it an attractive model for the development of biological adhesives for industrial and medical applications. Holdfast-mediated attachment to surfaces has been exploited to monitor large numbers of stalked *C. crescentus* cells in flow chambers for extensive periods of time, basically constituting one of the first, if not the very first, microfluidic experiments involving bacteria (Figure 4C). While stalked cells remain firmly attached to the walls of the flow chamber, a continuous flow of nutrients ensures constant growth conditions over time and removal of swarmer progeny once they detach after division. Following stalked cells of the same age over time (>150 generations) revealed that their reproductive output declined with increasing age, making *C. crescentus* the first bacterium reported to exhibit replicative aging (Figure 4C). Since then, similar phenomena have been described for symmetrically dividing

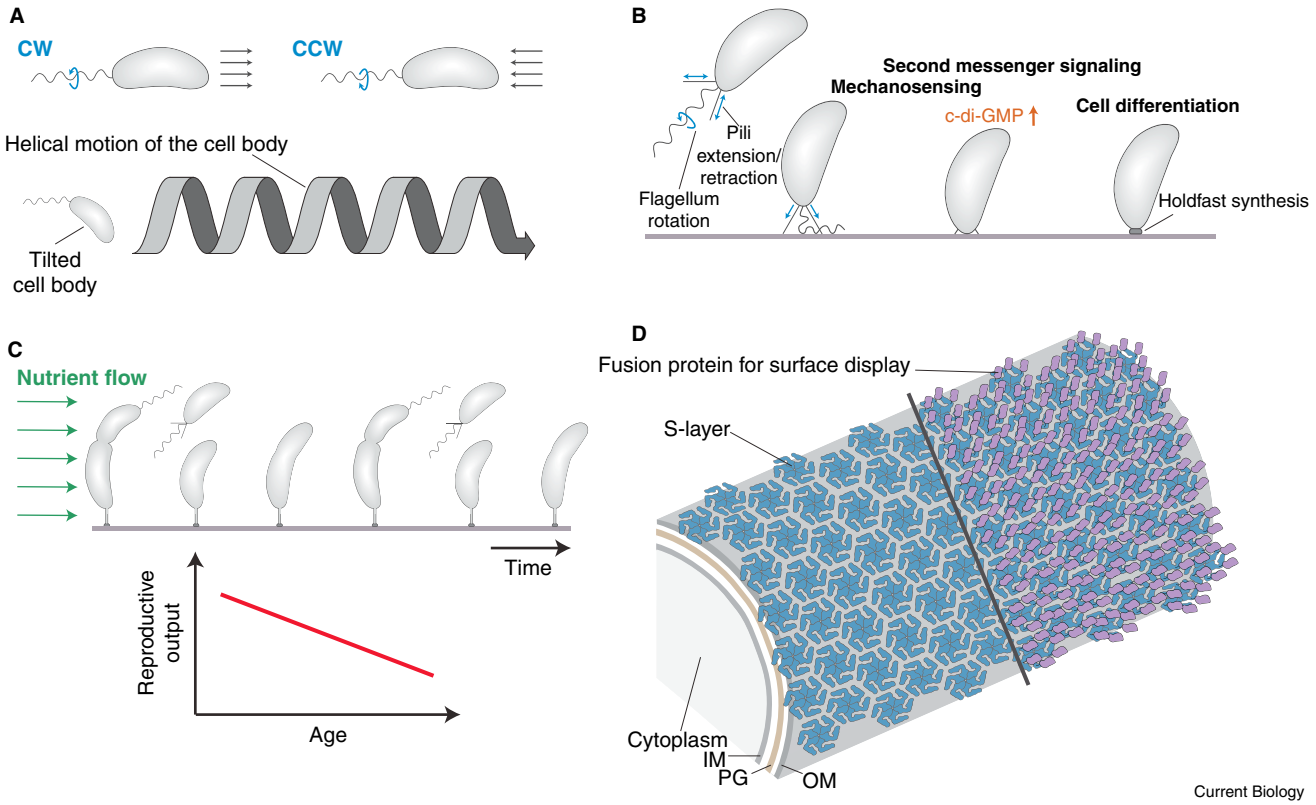

Figure 4. Diverse applicability of *C. crescentus* as a model system or tool.
(A) *C. crescentus* motility. *C. crescentus* cells move forward and backward by rotating their flagellum clockwise (CW) and counterclockwise (CCW), respectively. Motility is enhanced by the orientation of the cell body, which leads to a helical motion that generates thrust and increases swimming efficiency. (B) Mechanosensing in *C. crescentus*. Surface sensing through the pili and the flagellar motor leads to holdfast synthesis and cell differentiation through second messenger signaling. (C) Replicative aging in *C. crescentus*. Long-term monitoring of individual stalked cells in flow chambers revealed that their reproductive output decreases with cell age. (D) Protein surface display in *C. crescentus* through fusions to the RsaA S-layer protein. IM, inner membrane; PG, peptidoglycan; OM, outer membrane.

bacteria, in which replicative aging has been linked to pole age and old pole inheritance.

Another interesting aspect of *C. crescentus* physiology, from a biotechnological perspective, is its paracrystalline proteinaceous surface layer (S-layer). The *C. crescentus* S-layer is made of a single protein (RsaA) that is expressed to a reported level of ~30% of the cell's total protein content. Such S-layer proteins have become useful model systems for investigating the synthesis, secretion and assembly of extracellular proteins. A type I ABC transporter secretes RsaA to the cell surface, where it self-assembles into a two-dimensional hexagonal lattice bound to the O-antigen of lipopolysaccharides (Figure 4D). The RsaA S-layer has been used for both expression and surface display of peptides and proteins (Figure 4D). Fusion to the carboxy-terminal secretion signal of RsaA enables the production and secretion of heterologous proteins that are then easily retrieved from the growth medium. Fusion to the full-length RsaA protein has been used as a method for protein/peptide surface display for a variety of biotechnological applications, including biocatalysis, antigen display and bioremediation. Although similar approaches have been used for S-layer proteins of other species, the very first demonstration of S-layer-mediated surface display was a fusion of a heterologous peptide to RsaA, again highlighting the pioneering contributions of *C. crescentus*.

From innocuous bacterium to model system extraordinaire

It is clear that *C. crescentus* has come a long way, from a Californian pond to laboratories across the world, serving as a model system for bacterial cell biology, cell-cycle regulation, cell asymmetry, cell development, the evolution of morphological diversity, bacterial biophysics and biotechnology. During this process, *C. crescentus* has helped us challenge some of our most ingrained beliefs and changed the way we view bacteria. It has also paved the way for the exploration of other bacterial systems, serving as a gateway for increasing our understanding of other bacterial species with important roles in ecology, agriculture, human health and disease. If *C. crescentus* has taught us anything, it is that it is worth taking chances on inoffensive, seemingly odd organisms. The distinct features of *C. crescentus* have proven to be an apparently endless reservoir of new biological findings. With each new discovery, our understanding of the intricacy housed within this tiny organism increases and our appreciation of bacterial diversity grows.

FURTHER READING

Ackermann, M., Stearns, S.C., and Jenal, U. (2003). Senescence in a bacterium with asymmetric division. Science *300*, 1920.
Ausmees, N., Kuhn, J.R., and Jacobs-Wagner, C. (2003). The bacterial cytoskeleton: an intermediate filament-like function in cell shape. Cell *115*, 705–713.
Christen, M., Kulasekara, H.D., Christen, B., Kulasekara, B.R., Hoffman, L.R., and Miller, S.I. (2010). Asymmetrical distribution of the second messenger c-di-GMP upon bacterial cell division. Science *328*, 1295–1297.
Ingerson-Mahar, M., Briegel, A., Werner, J.N., Jensen, G.J., and Gitai, Z. (2010). The metabolic enzyme CTP synthase forms cytoskeletal filaments. Nat. Cell Biol. *12*, 739–746.
Jiang, C., Brown, P.J., Ducret, A., and Brun, Y.V. (2014). Sequential evolution of bacterial morphology by co-option of a developmental regulator. Nature *506*, 489–493.
Kiekebusch, D., Michie, K.A., Essen, L.O., Lowe, J., and Thanbichler, M. (2012). Localized dimerization and nucleoid binding drive gradient formation by the bacterial cell division inhibitor MipZ. Mol. Cell *46*, 245–259.
Kuhn, J., Briegel, A., Morschel, E., Kahnt, J., Leser, K., Wick, S., Jensen, G.J., and Thanbichler, M. (2010). Bactofilins, a ubiquitous class of cytoskeletal proteins mediating polar localization of a cell wall synthase in Caulobacter crescentus. EMBO J. *29*, 327–339.
Laub, M.T., McAdams, H.H., Feldblyum, T., Fraser, C.M., and Shapiro, L. (2000). Global analysis of the genetic network controlling a bacterial cell cycle. Science *290*, 2144–2148.
Le, T.B., Imakaev, M.V., Mirny, L.A., and Laub, M.T. (2013). High-resolution mapping of the spatial organization of a bacterial chromosome. Science *342*, 731–734.
Lim, H.C., Surovtsev, I.V., Beltran, B.G., Huang, F., Bewersdorf, J., and Jacobs-Wagner, C. (2014). Evidence for a DNA-relay mechanism in ParABS-mediated chromosome segregation. Elife *3*, e02758.
Lori, C., Ozaki, S., Steiner, S., Bohm, R., Abel, S., Dubey, B.N., Schirmer, T., Hiller, S., and Jenal, U. (2015). Cyclic di-GMP acts as a cell cycle oscillator to drive chromosome replication. Nature *523*, 236–239.
Quon, K.C., Marczynski, G.T., and Shapiro, L. (1996). Cell cycle control by an essential bacterial two-component signal transduction protein. Cell *84*, 83–93.
Radhakrishnan, S.K., Thanbichler, M., and Viollier, P.H. (2008). The dynamic interplay between a cell fate determinant and a lysozyme homolog drives the asymmetric division cycle of Caulobacter crescentus. Genes Dev. *22*, 212–225.
Sommer, J.M., and Newton, A. (1991). Pseudoreversion analysis indicates a direct role of cell division genes in polar morphogenesis and differentiation in Caulobacter crescentus. Genetics *129*, 623–630.
Willett, J.W., Herrou, J., Briegel, A., Rotskoff, G., and Crosson, S. (2015). Structural asymmetry in a conserved signaling system that regulates division, replication, and virulence of an intracellular pathogen. Proc. Natl. Acad. Sci. USA *112*, E3709–E3718.

[1]Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA.
[2]Department of Biology and ChEM-H Institute, Stanford University, Stanford, CA 94305, USA.
*E-mail: jacobs-wagner@stanford.edu

Primer

Plasmid evolution

Tanita Wein and Tal Dagan

Plasmids are genetic elements that colonize and replicate in prokaryotic cells (Box 1). They are considered a major driving force of prokaryote evolution, as they can migrate between populations, making them potent agents of lateral DNA transfer and microbial warfare. The importance of plasmids goes beyond microbial evolution, as they are widely used as vectors for genetic engineering in basic research (e.g., random mutagenesis) as well as applications in biotechnology (e.g., insulin production), synthetic biology, agriculture (e.g., genetic engineering of crops) and medicine (e.g., biopharmaceuticals).

Plasmids encode the mechanisms related to their replication and lateral transfer, and they often carry additional genes that encode diverse metabolic or physiological functions. Accordingly, plasmid acquisition may enable evolutionary and ecological expansion of bacterial populations. Examples are the contribution of plasmids to rapid adaptation in growth-limiting conditions (e.g., in the presence of antibiotics), or to long-term transitions in the bacterial life style (e.g., pathogenicity). Plasmids enable adaptation not only through the supply of new functions, but also through variation in their copy number that can lead to an elevated gene expression level and mutation supply rate (as shown recently by Rodriguez-Beltran *et al.* (2018)).

Although plasmids can potentially benefit their bacterial hosts, plasmid replication and gene expression utilizing the host machinery are considered a metabolic burden for the host. Thus, in the absence of selection for plasmid-encoded traits, plasmid-free cells are expected to outcompete plasmid-carrying cells, while the plasmid is doomed to extinction. Nonetheless, plasmids are ubiquitous in nature. Plasmid persistence in microbial populations is facilitated by a combination of several factors, including plasmid stability, plasmid mobility, the level of plasmid–host co-adaptation and the presence of plasmid genes that are beneficial for the host.

Plasmid stability and the plasmid life cycle

Plasmid persistence depends on plasmid replication and stable vertical inheritance (i.e., the plasmid life cycle; Figure 1). Plasmid replication is controlled by plasmid-encoded components, while the host cell provides the DNA replication machinery. The interaction with the host replication machinery is thus crucial for plasmid persistence in the host cell. Plasmid replication is divided into three main steps: replication initiation, elongation and termination. Essential features of plasmid replication are the origin of replication (*oriV*) that is typically flanked by specific initiator sites and genes encoding for proteins that function in the plasmid replication (Rep proteins).

Plasmid replication is initiated by an interaction between a plasmid replication primer and the host DNA polymerase. In the theta and strand-displacement plasmid replication types, the replication is initiated by

Box 1: What is a plasmid?

Darwin couldn't define a 'species' but said we all know what we mean by this term. The term 'plasmid' was coined by Joshua Lederberg in 1952, and our provisional understanding of a plasmid is that it is a genetic element that is *extra-chromosomal*, yet some plasmids are temporarily integrated into chromosomes; it is a small *circular* genome, yet some plasmids are linear; it is *mobile* or self-transmissible, yet about half of the known plasmids are non-mobilizable or not self-transmissible; it is transferred as *naked DNA*, yet some plasmids have their own packaging; it can have *positive or negative effect* on the host fitness, yet some plasmids are cryptic and have no clear impact on the host. Notably, the commonality among these plasmid features is an inherent bias that views plasmids primarily from the point of view of their host cells. This bias stems from the historical circumstances of their discovery, and from their great utility as laboratory tools.