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MINIREVIEW

Synchronized Swarmers and Sticky Stalks: *Caulobacter crescentus* as a Model for Bacterial Cell Biology

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ABSTRACT First isolated and classified in the 1960s, *Caulobacter crescentus* has been instrumental in the study of bacterial cell biology and differentiation. *C. crescentus* is a Gram-negative alphaproteobacterium that exhibits a dimorphic life cycle composed of two distinct cell types: a motile swarmer cell and a nonmotile, division-competent stalked cell. Progression through the cell cycle is accentuated by tightly controlled biogenesis of appendages, morphological transitions, and distinct localization of developmental regulators. These features as well as the ability to synchronize populations of cells and follow their progression make *C. crescentus* an ideal model for answering questions relevant to how development and differentiation are achieved at the single-cell level. This review will explore the discovery and development of *C. crescentus* as a model organism before diving into several key features and discoveries that have made it such a powerful organism to study. Finally, we will summarize a few of the ongoing areas of research that are leveraging knowledge gained over the last century with *C. crescentus* to highlight its continuing role at the forefront of cell and developmental biology.

KEYWORDS bacterial cell biology, *Caulobacter*, *Caulobacter crescentus*, model organism, cell cycle, differentiation, morphogenesis

In a tap in Chicago, there lived a bacterium. An object of fascination for many bacteriologists in the early 20th century, *Caulobacter* spp. were initially described as curved rod-shaped bacteria that formed immobilized rosettes of cells (Fig. 1). These bacteria bore "flagella-like" appendages, later termed "stalks," that extended into the center of these rosettes and divided transversely such that newly formed daughter cells were released. *Caulobacter crescentus* (here referred to as *Caulobacter*) is the primary model organism representing the family *Caulobacteraceae* in the order *Caulobacterales* (named from the Greek $\kappa\alpha\upsilon\lambda\text{o}\varsigma$, meaning "stalk"). This order was first proposed in 1935 by Henrici and Johnson (1) in their observational work describing the existence of a novel stalked bacterial species (previously, and independently, observed by Mabel Jones [Fig. 1] [2] and Vasily Omeliansky [3]) that had adhered to a slide submerged in lake and tap water. Houwink and van Iterson (4) and Bowers et al. (5) then demonstrated the presence of a singular flagellum by electron microscopy and presented an initial protocol for maintaining laboratory cultures. Finally, Jeanne Poindexter isolated and optimized a protocol for cultivation of several *Caulobacter* species from fresh water (6). These isolates included a strain from a pond in California in 1960 that would become the ancestor to the currently used CB15 and CB15N/NA1000 lab strains (7). These efforts set the stage to develop *Caulobacter* as a model organism more than 60 years after its initial description.

A striking feature of *Caulobacter* is its asymmetry. As a Gram-negative vibrioid bacterium, *Caulobacter* exhibits both lateral and longitudinal asymmetry; it has an inner and outer curvature as well as distinct poles that can possess either a flagellum or a stalk. Cell division results in two distinct daughter cells, a flagellated "swarmer cell" and a "stalked cell" bearing the aforementioned stalk, which reenter the cell cycle at

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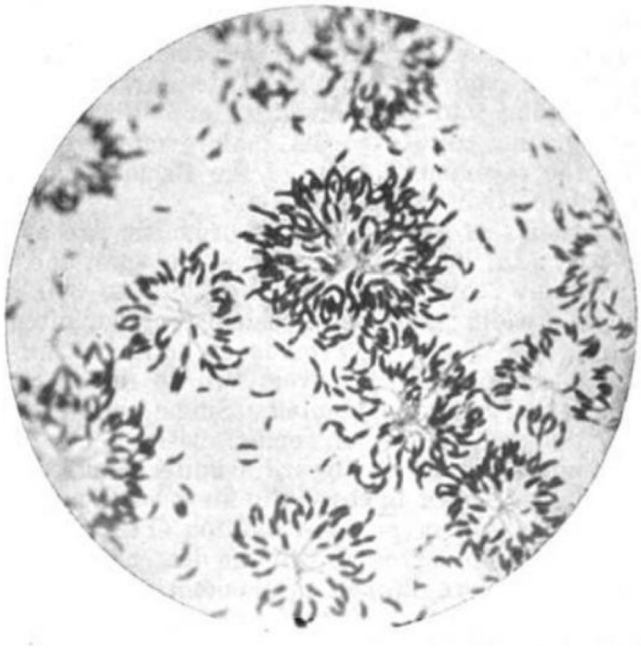

FIG 1 The first published image of *Caulobacter*-like cells. Film micrograph from 1905 depicting *Caulobacter*-like cells isolated by Mabel Jones from tap water in Chicago after 72 h in culture. Featured are rosettes of cells formed by descendants of single cells. Stalks are visible in the middle of rosettes, and predivisional and swarmer cells are both visible toward the periphery and in isolation. (Reproduced from reference 2).

different points (Fig. 2). This asymmetric division was observed from the outset, and its implication for the survival strategy of *Caulobacter* is nicely summarized by Henrici and Johnson: "It is probable that the outer most cell is set free after cell division, and either swims or floats away until a new substrate is encountered, when it proceeds to secrete a stalk" (1). Indeed, studies since then have confirmed that the swarmer cell is motile, driven by its flagellum until pili-mediated initiation of surface adhesion, after which production of holdfast at the pole secures attachment (8). The flagellum is then shed, and the former swarmer cell differentiates into a division-competent stalked cell, although this differentiation does not strictly require surface attachment, as *Caulobacter* strains deficient in surface attachment are still culturable and progress through the cell cycle (7, 9). The well-defined, morphologically distinct life cycle of *Caulobacter* was immediately recognized as providing a useful model for studying cell cycle progression, and that is where our story really begins.

THE DEVELOPMENT OF *CAULOBACTER* AS A MODEL ORGANISM

The study of any aspect of bacterial cell biology requires not only the ability to culture a bacterium of interest but also the ability to manipulate it to test hypotheses about its function. Recognizing the potential of *Caulobacter* to serve as a model for bacterial development, Lucy Shapiro outlined three necessary criteria for this kind of study: the organism must (i) have a simple and well-defined differentiation pattern, (ii) be culturable on defined media, and (iii) be genetically tractable. Shapiro proposed that *Caulobacter* exhibited all of these qualities, and its utility as a model was further potentiated by additional, as of yet unrecognized, technical advantages discussed below (10). Work with *Caulobacter* over the next 50 years, with significant initial contributions from the labs of Shapiro, Bert Ely, and Austin Newton, would lay the groundwork for the blossoming field of bacterial cell biology and establish *Caulobacter* as a powerhouse model organism for this type of study and beyond.

One of the exciting reasons for initially adapting *Caulobacter* as a model organism is the fact that it undergoes morphological differentiation throughout its cell cycle,

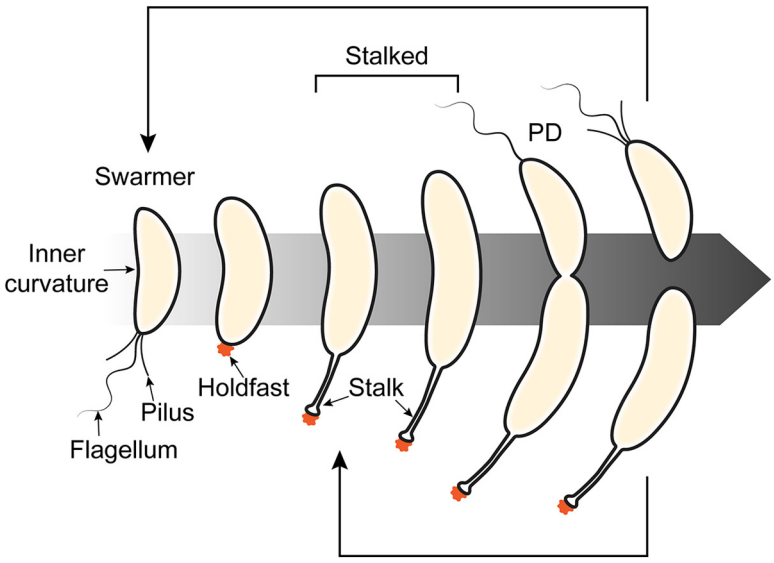

FIG 2 *Caulobacter* differentiates over the course of its cell cycle. A swarmer cell bearing a single flagellum and pili sheds or retracts these appendages and secretes holdfast (orange) coincident with differentiation to a stalked cell. The predivisional (PD) cell divides asymmetrically to yield swarmer and stalked daughter cells, both of which reenter the cell cycle at their respective stages. The new flagellum is formed concurrent with cell division, while pili are only detectable after completion of constriction.

unlike most bacterial models that had been studied up until that point. Along with the observations that genes are expressed at different levels at different times and in response to environmental stimuli, this suggested roles for regulated transcription, protein synthesis, and protein degradation as possible mechanisms for controlling progression through the cell cycle (10). To begin to answer questions about the details of this control, it was necessary to develop genetic tools to manipulate *Caulobacter* as well as methods for observing progression through the cell cycle and isolating cells at specific stages.

One of the first major innovations in the study of *Caulobacter* was the synchronization of cells with respect to cell cycle phase, arguably one of the most powerful tools for those who study the *Caulobacter* cell cycle. Synchrony relies on separation of cell types from one another, and protocols have evolved over time to increase both precision and yield. Cultures can be synchronized by one of (at least) two methods. Cultures can be incubated in glass petri plates to allow for adhesion of cells. Subsequent gentle agitation ensures that new swarmer cells are unable to adhere, allowing for their isolation from the culture medium (11). Alternatively, one can synchronize cells by subjecting a planktonic culture to centrifugation on a density gradient (12, 13), as stalked/predivisional cells synthesize a polysaccharide capsule that is absent in swarmer cells (14), allowing for separation by buoyancy (Fig. 3). After isolation, swarmer cells progress through the cell cycle synchronously, allowing for precise observation and measurement of cell cycle-dependent processes, which has made *Caulobacter* the premier organism for this type of analysis.

Following the ability to synchronize populations, the first breakthrough in beginning to understand the connection between genetic regulation and development was the isolation of *Caulobacter*-specific bacteriophages. The ϕCb5 RNA (15) and ϕCbk DNA (16) bacteriophages were found to specifically infect *Caulobacter* and to only do so during certain parts of the cell cycle. ϕCb5 requires the presence of flagellum-adjacent pili for adsorption, which are only present on swarmer and late predivisional cells (17). ϕCbK, however, binds specifically to pili (18), and preliminary evidence suggests that it then binds to an uncharacterized lipopolysaccharide (LPS) receptor site that arises coincident with the transition to stalked cells (10). These discoveries allowed for

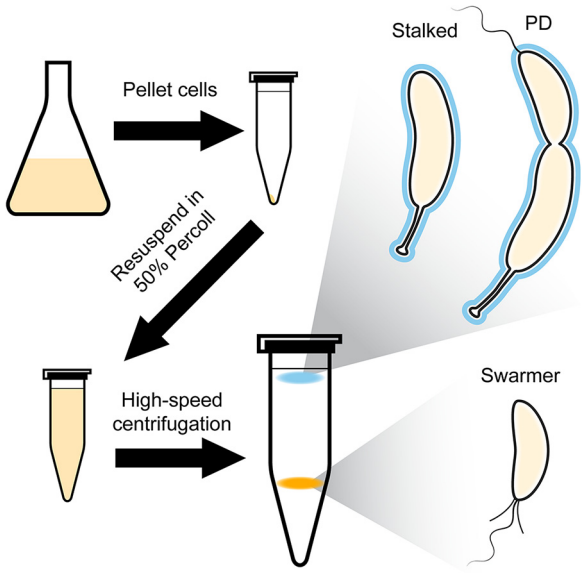

FIG 3 Schematic of swarmer cell isolation for synchrony of *Caulobacter* cultures. To isolate swarmer cells, a log-phase culture of *Caulobacter* is pelleted and resuspended in a solution of 50% Percoll in minimal medium, followed by high-speed centrifugation to segregate cells by buoyancy. Swarmer cells (orange band) are separated from stalked and predivisional (PD) cells (cyan band) due to a difference in buoyancy resulting from the production of capsule (cyan outline) in stalked and PD cells. The full protocol is available in reference 13.

the development of assays to follow progression through the cell cycle by infecting synchronized populations of *Caulobacter* with phage and observing the efficiency of infection over time, which resulted in the first approximation of the timing of each stage in the cell cycle (19). This innovation also allowed for the isolation of temperature-sensitive (TS) mutants that halted transition from one cell cycle state to another, opening the door for discovery of factors that are required for various stages of development (10). Suppressor screens using TS mutants allowed for the discovery of yet more crucial regulators of progression (20).

Further advancements in genetics (reviewed in reference 21) included the ability to generate and isolate spontaneous single mutants (22), the development of a conjugation system (23), a method for phage-based generalized transduction (24), the ability to transform a plasmid via electroporation (25), a method for chromosomal gene inactivation (26), and the use of the Tn5 transposon to integrate randomly in the genome without apparent bias (27). Finally, the *Caulobacter* genome map was constructed, first by a combination of conjugation and transduction (28) and later by restriction digest followed by pulsed-field electrophoresis (29). The entire genome sequence was published in 2001 (30). These advancements allowed for specific targeting of individual genes and regions, establishing a powerful and versatile genetic system that could be used to start answering questions about bacterial morphogenesis and cell cycle control.

After several years of work in multiple laboratories, a derivative of the original CB15 *Caulobacter* strain was isolated (called NA1000 or CB15N) that provided several technical advantages over CB15 (7). Genomic sequence analysis revealed several single nucleotide polymorphisms (SNPs), including a frameshift mutation in the holdfast synthesis gene *hfsA* and a mutation in the *zwf* promoter. Notably, NA1000 also carries a 26-kb mobile element harboring sequences with a lower GC content than the rest of the genome, indicating acquisition by horizontal transfer, and containing genes that are likely involved in capsule biosynthesis. As a result of these mutations, NA1000 exhibits faster growth, limited adhesion, and decreased lysis following infection with the ϕCr30 transducing phage compared to CB15. NA1000 also exhibits a lower

propensity for stalked and predivisional cells to form a pellet after centrifugation, greatly enhancing the effectiveness of synchrony protocols. The combination of all these phenotypic differences, especially increased synchronization efficiency, resulted in the adoption of NA1000 as the primary *Caulobacter* strain used for research that does not require adhesion, and it remains the foremost studied strain today.

Beyond its utility as a model for differentiation, *Caulobacter* has also been a foundational model for the development and application of innovations in imaging. Fluorescence-based visualization of cells and subcellular components has been used since the invention and subsequent demonstration of immunofluorescence in 1941 and 1942, respectively (31, 32). A major improvement on this technique that allowed for visualization of proteins in live cells was the generation of fluorophore fusion constructs wherein a fluorescent protein is genetically conjugated to one of the termini of a protein of interest. One of the first demonstrations of this technique in bacteria was in *Caulobacter*, where Jacobs et al. demonstrated the localization of the histidine kinase CckA, which was tagged at its native locus with green fluorescent protein (GFP) (33). They found not only that it localizes to specific poles but also that its localization is dynamic over the course of the cell cycle. As another example of fluorescence-informed dynamic investigation, the first comprehensive study of bacterial chromosome organization was performed in synchronized populations of *Caulobacter* using a combination of fluorescence *in situ* hybridization and the fluorescence repressor operator system (34). This approach revealed that the positioning of chromosomal loci is stereotyped and that the segregation of the new chromosome following replication is tightly controlled with highly reproducible rates of movement and subcellular destinations for each locus.

The development of superresolution techniques has allowed for even further refinement of localization studies (35) and the investigation of the finer details of large cellular structures, such as the cytokinetic ring or Z-ring (36). Pushing the frontiers of imaging technologies, *Caulobacter* was one of the pioneer organisms in the development of electron cryotomography (cryo-ET) for probing fine structures and the three-dimensional organization of intact, unfixed bacterial cells with high resolution. *Caulobacter* was uniquely suited for this role early in the development of cryo-ET, as its cells are smaller than those of other well-studied bacterial models (e.g., *Escherichia coli* and *Bacillus subtilis*), allowing for visualization of cells in their entirety at high resolution (37). The first of these studies revealed that *Caulobacter* division concludes with constriction and fission events of the inner and outer membranes that are spatially and temporally distinct (38). Another demonstrated the presence of multiple classes of large filament bundles present in different orientations in *Caulobacter* cells (39). Ring-like and inner curvature-associated filaments were thought to be associated with the previously known cytoskeletal proteins FtsZ, MreB, and CreS, and these structures have since been studied in more detail to gain insight into their respective structures throughout the cell cycle. The structure of FtsZ filaments and their relationship to constriction were characterized in more detail using the unique morphology of a *Caulobacter* mutant overproducing the GTPase-deficient G105S FtsZ variant (40, 41).

Finally, as has become especially apparent in recent years with the exponential accumulation of data, the intimate study of a model organism is greatly aided by the existence of comprehensive databases. The first database-style resource for *Caulobacter* resulted from a high-throughput imaging screen to determine the distributions of nearly three-fourths of all *Caulobacter* proteins fused to fluorescent markers. This work yielded the *Caulobacter crescentus* localisome website, where users can find localization data tied to corresponding images for proteins of interest (42). Inspired by the recent acquisition of multiple "-omics"-level data sets, Lasker et al. endeavored to create CauloBrowser, a user-friendly, integrated online database that allows researchers to search for genes to gain information on their localization, expression profiles, interactions, etc. (43). Another important resource is the Fitness Browser, a multiorganism database that includes data on the relative fitness of various mutants under different conditions that allows users to gain insight on potential phenotypic effects and interactions among genes and proteins of

interest (44). The development of these and other databases represents a significant step forward in the ability to easily integrate information to generate new hypotheses.

Caulobacter has a long and storied past, and its utility as a model for the study of dynamic, cell cycle-dependent processes has been demonstrated time and time again. For the remainder of this review, we will explore some of the notable features of and discoveries in *Caulobacter* that have made this such a powerful organism to investigate.

UNIQUE FEATURES AND NOTABLE DISCOVERIES

The right tool for the job. Throughout its life cycle, *Caulobacter* uses distinct appendages to adapt and thrive under variable environmental conditions. Motile swarmer cells bear a single flagellum and pili at one pole. These cells can explore their environments then shed the flagellum and retract their pili, attach to a surface via the secretion of holdfast, and begin to differentiate into a stalked cell (Fig. 2). Stalked cells are competent for DNA replication, which occurs once per cell cycle (45), as well as division, which results in two morphologically and developmentally distinct daughter cells. The swarmer daughter can swim away and find a new environment in which to differentiate, and the stalked daughter immediately reenters the cell cycle at the point of DNA replication and cell division (Fig. 2).

The stalk has been noted from the earliest observations of *Caulobacter*. While early observers concluded that it might be a "flagella-like" appendage (1), transmission electron microscopy (TEM) experiments revealed that the stalk is an extension of the cell envelope, including both inner and outer membranes as well as peptidoglycan cell wall (4). Further observations suggested that the stalk is a biochemically distinct compartment, as evidenced by a lack of ribosomes and nucleoid and the presence of electron-dense crossbands along the stalk length (46). These crossbands create diffusion barriers that prevent exchange of proteins between the cell body and the stalk and between stalk compartments (47). Although the "swarmer-to-stalked" transition is a crucial step in the *Caulobacter* life cycle, the formation of the stalk is not strictly required for division to occur (48), suggesting that it functions in tandem with, but not as a regulator of, cell cycle progression.

The role of the stalk has been studied extensively in the decades since *Caulobacter* was isolated. Although holdfast, a strongly adhesive polysaccharide, is present at the tip of the stalk, holdfast synthesis precedes stalk biogenesis (49), and attachment is undertaken by swarmer cells, suggesting that the stalk does not play a role in surface attachment (46). Additionally, some stalked bacteria produce holdfast and stalks at separate locations (6), further indicating that surface attachment is not the stalk's primary function. The stalk appears to have a role in sensing or responding to nutrient availability (50), as *Caulobacter* cells starved of phosphate grow elongated stalks (51, 52). This was initially hypothesized to be a mechanism for increasing surface area to facilitate nutrient uptake (53), but this model has been challenged by the apparent lack of protein diffusion along the stalk. A more recent hypothesis suggests that stalk elongation would allow for cells within a surface-attached biofilm to extend their cell body beyond the surface of the biofilm, increasing exposure to a more nutrient-rich medium (54). However, this model is difficult to apply to organisms like *Asticcacaulis* spp., where the site of adhesion is not located at the site of stalk extension (6, 46), suggesting that the role of stalks is more complicated than can explained by a single model (55). Finally, the stalk-localized protein StpX, which is required for proper stalk elongation, appears to protect cells from Zn^{2+} toxicity and plays a role in storage and utilization of Cu^{2+} (55). Additional studies in diverse organisms with stalks and stalk-like appendages are required to shed light on their function(s).

The same TEM experiments that granted insight into the structure of the *Caulobacter* stalk also allowed for initial observation of its flagellum (4). *Caulobacter* swarmer cells possess a single flagellum that is shed as the cell transitions to a stalked cell (Fig. 2). This flagellum is similar in structure to other canonical bacterial flagella and is composed of a stator complex, a hook, and a filament, all of which are visible by TEM (56). Flagellar gene

expression is regulated in tandem with cell cycle progression, with genes falling into one of four different classes. Class I genes include global cell cycle regulators (e.g., *dnaA* and *ctrA*), which will be discussed in detail in a later section. These factors control expression of class II genes (57–59), which in turn regulate the expression of class III and IV genes, which encode structural components of flagella (reviewed in reference 60). Flagellins, the major component of the flagellar filament, are synthesized approximately 30 to 40 min before cell division in synchronized populations (61). Flagella are shed in swarmer cells before differentiation into stalked cells and can be observed in media or on surfaces previously occupied by cells (61).

At the flagellated pole, *Caulobacter* also synthesizes multiple type IV tight adherence (tad) pili, which are used for sensing and adhering to surfaces, are retracted following differentiation into stalked cells, and are synthesized once again on swarmer daughter cells following division (Fig. 2) (62). Tad pili, composed of a basal complex and a filamentous fiber of the pilin PilA, are dynamic, undergo cycles of extension and retraction, and are common across both bacteria and archaea. Surprisingly, extension and retraction of the pili are both facilitated in *Caulobacter* by a noncanonical, bifunctional ATPase motor CpaF (63).

Both flagella and pili mediate surface attachment and mechanosensation that trigger production of holdfast at the flagellated pole (64, 65). This signaling is primarily mediated by the second messenger 3′,5′-cyclic-di-GMP (c-di-GMP), which is present at low levels in swarmer cells and is synthesized in response to mechanical perturbation of the flagella and pili, activating pilus retraction and holdfast synthesis to facilitate adherence (66–68). Components of the flagellar machinery also mediate pilus biosynthesis, as loss of the former results in attenuated pilus formation (69). Additionally, transcription of the holdfast synthesis inhibitor gene *hfiA* is regulated both by flagellum assembly and nutrient availability (70, 71), providing a link between environmental cues and control of surface attachment.

Finally, like many other bacteria, *Caulobacter* cells are enveloped by a periodic proteinaceous S-layer that is continuously present throughout all stages of the cell cycle (72, 73). The sole S-layer protein of *Caulobacter*, RsaA, is continuously produced throughout the cell cycle and is homologous to S-layer proteins from other organisms (74). RsaA forms a hexagonal lattice structure that encompasses the entire cell and binds to lipopolysaccharide, as observed by high-resolution cryo-ET that was aided by the thin diameter of *Caulobacter* stalks (75, 76). Proposed functions of the S-layer in various bacterial species include adhesion to surfaces and protection against chemical and biological threats (reviewed in reference 77). Additionally, the transducing phage ϕCr30 requires the S-layer for adsorption and subsequent infection, indicating the presence of more complicated roles in the environment and for *Caulobacter* evolution (78).

A living integrated circuit. Most bacteria possess numerous mechanisms for exerting both spatial and temporal control, but this task is exquisitely complicated in a bacterium like *Caulobacter* that undergoes differentiation throughout its cell cycle. As we have explored so far, progression through the cell cycle is dependent on physical and chemical cues in the environment as well as internal timing. Moreover, in *Caulobacter*, cell cycle events are intimately coupled to polar development; but how are each of these steps regulated? *Caulobacter* possesses four global regulators (DnaA [79], GcrA [80], CtrA [81, 82], and CcrM [83]) that are produced sequentially and out of phase with one another (84) (Fig. 4) and together regulate over 200 genes required for progression to the next stages of the cell and developmental cycle, including each other (reviewed in references 85–87). Discovery of the extent of their regulatory networks began with development of the first *Caulobacter* microarray, made possible by the acquisition of the complete sequence of the genome (30). In this landmark study, temporal microarray measurements revealed that gene expression is coordinated with the function of respective gene products (e.g., expression of DNA replication machinery and divisome components is activated before their respective events in the cell cycle) (88). This study also revealed that the expression of genes whose products make up complexes are

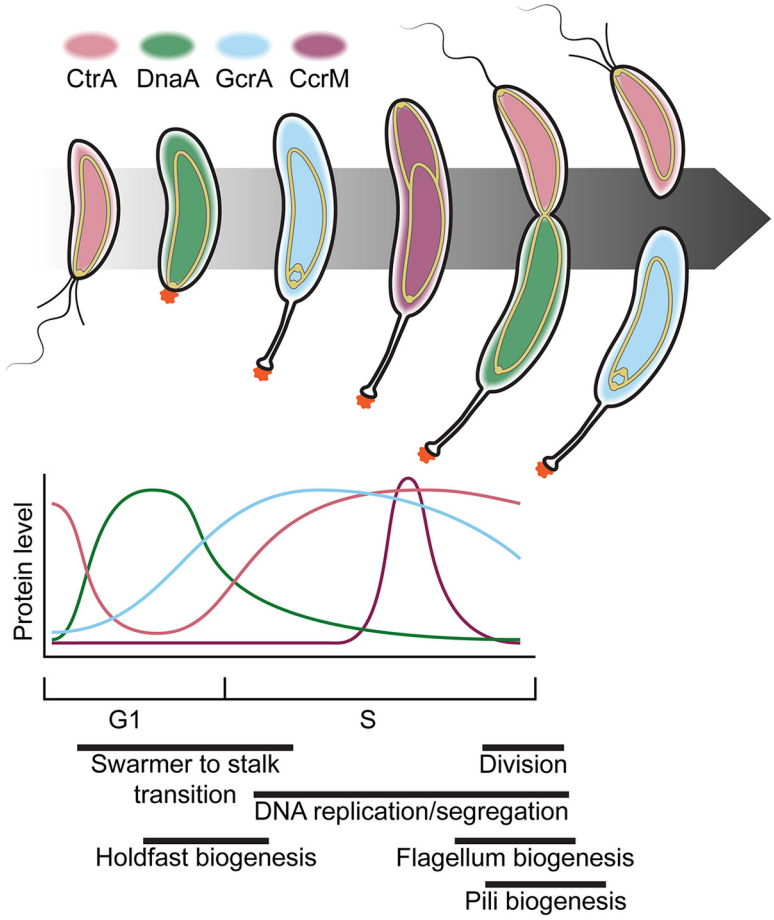

FIG 4 *Caulobacter* differentiation is mediated by global regulators. Progression through the cell cycle is shown as in Fig. 2, with the most abundant global regulator (CtrA, pink; DnaA, green; GcrA, cyan; CcrM, plum) present for each cell type indicated by the color of the cell. Relative levels of each global regulator throughout the cell cycle are shown in the graph below. The chromosome, shown as a gold-outlined ellipse (origins are indicated by circles at the ends), is anchored to the flagellar pole in swarmer cells (G_1 phase) and begins replication after the transition to a stalked cell (S phase). The origin of the newly replicated DNA is translocated to the nascent swarmer pole, and the terminus is directed to midcell, where the two chromosomes are distributed between the daughter cells coincident with cell division. Relative timescales of various morphogenetic events are indicated below (94, 101, 192).

typically coregulated and that regulation of biogenesis of large structures is regulated by integrated signal cascades. More recent work has suggested that the global integrated circuit that controls cell cycle progression includes genetically separable subcircuits that control distinct aspects of the cell cycle, such as CtrA/MucR in swarmer cells (89), GcrA/CcrM and DnaA during DNA replication (90, 91), and CtrA/SciP during cell division (92) (reviewed in reference 93). Further studies implicated global regulation of translation (94), phosphorylation (33, 95–98), and protein degradation (96, 99) as well as localization and production of small-molecule second messengers (100) as additional mechanisms of cell cycle regulation. These findings made it clear that *Caulobacter* acts as a biological integrated circuit, complete with interacting positive and negative cues that give rise to both temporal and spatial regulation (95, 101).

The CtrA protein is a response regulator that controls expression of over one-fourth of cell cycle-regulated genes, including those involved in flagellum and pilus biogenesis, DNA replication and methylation, signaling, and cell division (88). Phosphorylated CtrA (CtrA~P) inhibits replication initiation (102) and *gcrA* expression (84) in swarmer cells by binding to the origin of replication and the *gcrA* promoter, respectively. CtrA~P is later dephosphorylated and degraded at the stalked pole, allowing the

replication initiator DnaA to bind to the origin, resulting in initiation of replication and production of GcrA (reviewed in reference 103). Following initiation, DnaA activity is inhibited by HdaA (104), which stimulates conversion of DnaA-ATP to DnaA-ADP, to prevent reinitiation, and DnaA-ATP is regenerated via degradation of DnaA-ADP and production of newly synthesized DnaA (90) (reviewed in reference 105). DnaA is primarily degraded by Lon protease in the event of nutrient limitation or proteotoxic stress (106, 107) and by ClpXP or ClpAP under starvation conditions (106) or during the stationary phase (108), respectively, to ensure cell cycle arrest under stress (also summarized in reference 105). GcrA, a cofactor of the σ^{70} transcription factor (80), activates the expression of *ctrA* in its hemimethylated state (i.e., only when the replication fork has passed and before the newly synthesized strand is methylated) (81, 109). Newly synthesized CtrA then regulates its own expression to rapidly increase CtrA levels in the cell. CtrA∼P drives expression of *ccrM*, which encodes the methyltransferase CcrM that methylates the newly replicated genome, shutting down *ctrA* (81, 109) and *ccrM* (110) expression.

Cell identity is tightly controlled by an asymmetric logic system that integrates with the regulatory mechanisms described above. An overarching principle of this system is the dynamic interplay of interconnected signaling networks that work in tandem to control both pole identity and progression through the cell cycle (reviewed in references 82 and 111). The first component of this system that was discovered was *pleC*, the mutation of which resulted in loss of stalks and phage receptors as well as inactive flagella (46). The *pleC*-mutant phenotype provided a foundation for the discovery of other cell cycle regulators, including PleD (112), DivJ, DivK, and DivL (113) by isolation of bypass mutants that restored either motility or phage susceptibility to *pleC*-mutant cells. Localization and activity of the diguanylate cyclase PleD are regulated by its phosphorylation state. The phosphatase PleC inactivates PleD at the swarmer pole, and the kinase DivJ activates PleD at the stalked pole (114, 115) where it synthesizes c-di-GMP to signal for shedding of the flagellum and transition from swarmer to stalked cells (115–117).

The response regulator DivK provides a link between cell cycle progression and cell fate determination pathways. Like PleD, DivK is phosphorylated by DivJ at the stalked pole and dephosphorylated by PleC at the swarmer pole in predivisional cells. This results in differential localization of phosphorylated DivK (DivK∼P) and DivK in the stalked and swarmer compartments, respectively, as division proceeds (118, 119). DivK∼P inactivates DivL, a pseudokinase that activates the hybrid histidine kinase CckA, which phosphorylates and activates CtrA (120–122). Chromosome segregation is also required for this process, as the histidine phosphotransferase ChpT (93) is only recruited to the swarmer pole after successful segregation. ChpT localization completes the CckA-ChpT-CtrA phosphorelay to activate CtrA signaling and allow for progression through cell division (123). High levels of c-di-GMP produced by PleD in the stalked compartment (124) induce CckA to dephosphorylate CtrA (97, 125, 126) and lead to CtrA degradation by ClpXP (127). Swarmer pole identity is reestablished by the polar marker TipN (128, 129), which localizes to the nascent swarmer pole and recruits the phosphodiesterase TipF (flagellar assembly [128]) and PleC (48, 129), allowing for the relocalization of swarmer cell determinants and flagellar and pili assembly machinery (130).

A model for progress. *Caulobacter* is a premier bacterial model for studying cell cycle progression. The presence of morphological markers and clearly defined asymmetry at every step in the cell cycle means that cells at any point in the cell cycle can be readily identified, even in unsynchronized populations. This, along with the ability to isolate swarmer cells and follow their progression, allows for powerful analysis of events that are coordinated with the cell cycle.

The *Caulobacter* cell cycle includes several distinct stages (Fig. 2). One of the key developmental stages in the *Caulobacter* life cycle is the differentiation from a swarmer cell to a stalked cell, which is coincident with entry into S phase. As one would predict based on

the necessity of available nutrients for propagation, the swarmer-to-stalked (and G_1 to S) transition is slowed under nutrient limitation. After starvation, *Caulobacter* initiates the stringent response, mediated by the small molecule alarmones (p)ppGpp, the synthesis of which delays this transition and regulates expression of numerous genes involved in metabolism, genome replication, growth, and division (131–133).

In the presence of nutrients, swarmer cells transition to a stalked fate and enter S phase, which is marked by replication and simultaneous segregation of the chromosome. This occurs exactly once per cell cycle in logarithmically growing cells, and its regulation is tightly coordinated with cell division. The *Caulobacter* origin of replication is anchored to the stalked pole via indirect interactions with the polar organizing protein PopZ (134, 135) and is acted upon primarily by the replication activator DnaA and the response regulator CtrA, as described in the previous section. PopZ is a scaffolding protein containing an intrinsically disordered region (IDR) and structured regions that together modulate interactivity (136, 137). PopZ forms a biomolecular condensate to create ribosome-free microdomains at the poles that selectively regulate entry of CckA, the phosphotransferase ChpT, and CtrA to regulate and enhance the phosphosignaling underlying cell differentiation (138, 139).

Replication continues until the genome is completely duplicated, during which a single origin remains at the stalked pole, while the second origin is translocated to the nascent swarmer pole by the *parABS* system (Fig. 5A). ParB is a DNA-binding CTPase that recognizes and binds to the origin-adjacent *parS* sequence, forming a ParB complex that spreads along the DNA (140). ParB binds to ParA, an ATPase that binds sequence nonspecifically to the chromosome, and regulates its nucleotide cycle to establish a ParA-ATP gradient (141–143). The combined biochemical activities and interactions of ParA and ParB result in processive diffusion of one copy of the ParA-ParB-*parS* complex along the length of the predivisional cell to the nascent swarmer pole, with regulatory input from TipN and PopZ (142, 144, 145). PopZ captures the newly segregated ParB-*parS* complex and anchors it at the swarmer pole (134, 135). The *parABS* system is broadly conserved for both genome and plasmid partitioning, having been identified in 69% of bacterial genomes surveyed (146), and the insights gained from the study of chromosome segregation in *Caulobacter* have been instrumental in establishing a mechanistic paradigm for its function.

Cell division, the final major step in the *Caulobacter* cell cycle, is unsurprisingly closely linked with DNA replication and segregation. This is important for two reasons: (i) to ensure that each daughter cell contains a copy of the genome and (ii) to ensure that the genome does not interfere with the process of constriction. Constriction activation begins with the tubulin homolog FtsZ, which localizes approximately to midcell to form a ring-like structure marking the division plane (Fig. 5, right) (147, 148) (reviewed in reference 149). FtsZ then recruits downstream components of the divisome, a multiprotein machinery responsible for performing cell division, including cell wall synthases that modify the cell wall to drive a change in shape that eventually results in constriction (150).

FtsZ localization to midcell is crucial for proper cell division to occur and is driven by interactions with MipZ, which binds to ParB and interferes with FtsZ polymerization (Fig. 5A) (151). MipZ forms a gradient emanating from the poles, resulting in an area of low concentration at midcell, allowing for FtsZ polymerization and the formation of the Z-ring there. Importantly, production of several divisome factors, including FtsZ, is tightly regulated throughout the cell cycle. The expression of *ftsZ*, inhibited by CtrA∼P, increases coincident with differentiation into a stalked cell, and FtsZ levels peak just before the start of division (152). Following the initiation of constriction, FtsZ is degraded rapidly by ClpXP and ClpAP (153, 154), leading to clearance in the nascent swarmer cell and maintenance of low levels of FtsZ in the stalked cell until the next round of cell division (152). Like in the case of DNA replication, tight regulation of divisome components ensures that division only occurs at the correct time in the cell cycle (i.e., after DNA replication and cell differentiation have progressed sufficiently). Interference of envelope constriction by the nucleoid is also regulated by the protein FtsK. FtsK is composed of two domains, a C-terminal DNA translocation domain and an N-terminal transmembrane domain associated with activation of cell wall

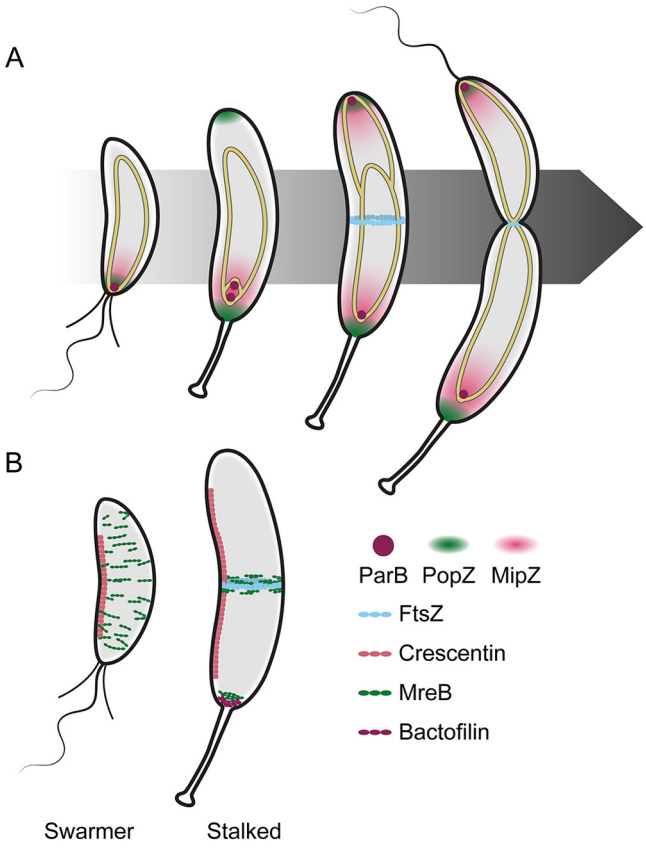

FIG 5 Spatial regulation of morphogenesis in *Caulobacter*. (A) FtsZ localization is driven by interactions with the chromosome (gold-outlined ellipse). ParB (plum circle) binds the *parS* sequence near the origin (circles at the ends of the chromosomes) and associates with PopZ (green cloud) at the pole. ParB translocates with the newly synthesized origin and anchors it to the nascent swarmer pole (top of cells). MipZ (pink cloud) binds to ParB and to the DNA to form a gradient, limiting FtsZ (cyan) polymerization to midcell in stalked and predivisional cells. (B) Localization of four major cytoskeletal proteins (crescentin, pink; MreB, green; FtsZ, cyan; bactofilin, plum) shown in swarmer (left) and stalked (right) cells. Crescentin directs curvature in both cell types. MreB directs elongasome localization and global cell wall insertion in swarmer cells and midcell insertion in stalked cells to facilitate elongation. FtsZ directs divisome assembly and cell wall remodeling at midcell in stalked cells to facilitate constriction. Bactofilins and MreB recruit and regulate stalk elongation machinery during the swarmer-to-stalk transition and remain localized at the base of the stalk in stalked cells.

synthases. Both domains are essential for proper chromosome segregation and constriction, suggesting that it serves as another link between these processes to ensure timely completion of segregation before the completion of constriction (155, 156).

Following FtsZ localization to midcell, the rest of the divisome must assemble, and many of these recruitment events are dependent on the correct localization of upstream factors. This epistatic pathway has been observed in other bacteria, but the actual timing of each component's arrival is difficult to ascertain without the ability to synchronize a population of cells. Leveraging *Caulobacter* synchrony to this end, Goley et al. observed the timing of midcell localization of roughly 20 factors involved in *Caulobacter* division (157) with a level of precision that was previously unavailable, giving critical insight into the timing of divisome assembly and its regulation.

Shape matters. As one would expect in a vibrioid, asymmetric bacterium, *Caulobacter* encodes numerous factors that play a role in morphogenesis and shape determination (reviewed in reference 158). The divisome, described above, is the most broadly conserved morphogenetic system, primarily controlling cell length (reviewed in reference 150). The next system is known as the elongasome, organized by the actin-like protein MreB, which associates with the Rod complex to guide cell wall insertion and drive elongation, generating the cell's rod-like shape (159). Unlike FtsZ, however, MreB levels are relatively constant

throughout the cell cycle (88, 94), consistent with the notion that the elongasome functions constantly during cell growth. MreB filaments assemble along the inner membrane perpendicular to the long axis of the cell, resulting in dynamic, broadly distributed patches early in the cell cycle that are localized to midcell in an FtsZ-dependent manner before division (Fig. 5B) (159, 160). The divisome and elongasome are conserved in many other species, which has allowed for the undertaking of comparative studies to understand how these systems function in species-specific manners.

Both the divisome and the elongasome act on the peptidoglycan cell wall, with cytoskeletal proteins directing construction and modification of this rigid macromolecule to effect changes on overall cell shape (reviewed in reference 161). Similarly, the curvature of *Caulobacter* is mediated by crescentin (encoded by *creS*), the first intermediate filament-like protein described in bacteria. Crescentin assembles into curved filaments along the inner curvature of the cytoplasmic membrane and is required for cell curvature (Fig. 5B) (162). Crescentin is thought to exert its effects on cell shape by mechanically regulating cell wall insertion such that more cell wall synthesis occurs distal to crescentin localization than occurs proximal to crescentin. Ultimately this asymmetry in cell wall synthesis yields a curved cell shape. Curvature has been associated with increased motility in bacteria (163) (e.g., in *Vibrio cholerae* [164]). Findings that *Caulobacter* exhibits low flagellar motor torque (165) and helical body movement (166), both of which result in increased swimming efficiency, suggest that this is a key adaptation for a microbe that inhabits low-nutrient environments. Curvature has also been observed to play a role in *Caulobacter* adhesion to surfaces when dividing under flow (167). The discovery of crescentin inspired investigation into the shape determinants in other vibrioid cells, and, indeed, proteins such as CrvA in *V. cholerae* appear to perform a similar function with a similar proposed mechanism (168).

Like the systems described above, stalk morphogenesis is partly facilitated by cytoskeletal proteins. Bactofilins, which were first described in *Caulobacter*, are widely conserved, bacterium-specific cytoskeletal proteins that form membrane-associated polymers or sheets with a variety of functions across species (169). The *Caulobacter* bactofilins, BacA and BacB, accumulate at the nascent stalked pole before stalk formation and are continuously present at the base of the stalk (Fig. 5B, right). These bactofilins interact with the cell wall synthase PbpC, which is required for the recruitment of the stalk elongation modulator StpX (55, 170), and recruit it to the stalk. The absence of any of these factors results in a decrease in stalk length but do not change overall stalk structure, indicating roles specifically in stalk extension (171). MreB and RodA, components of the elongasome, also localize to the base of the stalk (Fig. 5B, right) and are necessary for stalk formation, as depletion of either protein results in a stalk elongation defect (172). Further studies implicated the autolytic enzymes DipM, SdpAB, and CrbA, which are also associated with divisome function, and LdpA, the gene of which lies in an operon with *bacA*, in stalk morphogenesis. MreB inhibition results in a failure of each of the autolytic enzymes, but not BacA, to localize to the stalked pole. Although the ultimate determinant of polar localization for these complexes remains unknown, these interactions suggest a mechanism involving the combined regulation of multiple scaffolding proteins and cell wall remodeling complexes for effecting stalk formation and elongation (173). Finally, the aforementioned crossbands that limit diffusion along the length of the stalk are composed of a complex of four proteins, StpABCD, with StpA directing the recruitment of the rest of the complex (47). Stalk morphology in related stalked organisms (e.g., *Asticcacaulis* and *Brevundimonas*) (46, 174) resembles that of *Caulobacter*. However, while BacA, the elongasome, and hydrolytic enzymes are generally well conserved in this group, several stalk-related genes, including *bacB*, *pbpC*, *stpX*, and *stpABCD*, are not well conserved outside *Caulobacter*, suggesting differences between the mechanisms of stalk morphogenesis among these species (175).

CURRENT/FUTURE QUESTIONS

While the bulk of this review has focused on the features and discoveries of *Caulobacter* that have led to its establishment as one of the premier model organisms

for bacterial cell biology, we would be remiss if we failed to highlight some of the ongoing work and future directions for this exciting microorganism. One of these areas is the study of *Caulobacter* spp. in their native environments, taking the knowledge that we have acquired in the laboratory and applying it to understanding how the niche(s) that *Caulobacter* spp. occupy shape its biology (176, 177) and how *Caulobacter* spp. have evolved to interact with other organisms in those environments. Recent findings indicate that *Caulobacter* is far more abundant in soils than aquatic environments, in contrast to early predictions about the environmental range of *Caulobacter*, demonstrating its ability to survive in oligotrophic environments and indicating roles in decomposition and dispersal dynamics (178). Additional studies have showcased the discovery of multiple novel *Caulobacter*-targeting phages, including the first lysogenic phage found for *Caulobacter* to date (179), suggesting that there is a great deal more to discover about *Caulobacter* and its interaction with phages. Another phage was isolated from the rhizosphere, corroborating previous reports indicating that some *Caulobacter* species can localize to regions of plant growth and could fulfill roles as plant growth-promoting bacteria (180). There are numerous open questions about the role(s) that *Caulobacter* plays in these communities and how those roles might depend on features specific to a dimorphic, differentiating bacterium.

Many of the morphological features and subcellular complexes studied in *Caulobacter* are conserved across *Alphaproteobacteria*, making *Caulobacter* a useful model for engaging in comparative morphology and evolutionary studies to determine how these systems arise. Combining genomic sequence data with high-throughput culturing and imaging methods, it is possible to study the evolution of various morphological phenomena (e.g., polar growth, stalk formation, etc.) and identify the molecular determinants underlying their development (174). Having a well-studied, easily tractable organism like *Caulobacter* in hand allows for the generation of hypotheses about how various morphological systems evolved and are regulated across species. For example, our understanding of stalk formation and development in *Caulobacter* aided in the evolutionary and morphogenetic analysis of stalk placement and biogenesis in two *Asticcacaulis* species that form nonpolar stalks, resulting in the discovery of a novel function for the developmental regulator SpmX in these species (181).

The condensate-forming protein PopZ has promising future applications in the study of cell biology and biophysics. A PopZ-based recruitment assay (analogous to bacterial two-hybrid assays) was recently developed to enable the study of exogenous protein-protein interactions using *E. coli* as a host (182). Another study has recently indicated the potential of PopZ as a tunable model for generating synthetic condensates for the investigation of general properties *in vitro* and in mammalian cells (136).

Outside the field of cell biology, *Caulobacter* and its relatives are powerful organisms for the development of new technological advances. *Caulobacter* holdfast has been measured among the strongest known biological adhesives (183), and holdfast from the closely related *Hirschia baltica* exhibits an even greater degree of adhesion via an increased tolerance to ionic strength (184, 185), presenting opportunities for the development of versatile medically or commercially relevant adhesives (186). The *Caulobacter* S-layer is also being developed as a platform for synthesis and secretion of novel materials. The first study to demonstrate the feasibility of using *Caulobacter*'s S-layer secretion system to produce recoverable recombinant proteins was published in 1997 (187). This innovation, in combination with efforts to characterize S-layer assembly (188) and *Caulobacter* behavior and adaptability in a dense biofilm setting (189), has led to the development of a robust system for generating engineered living materials that have potential applications in human health, energy, and the environment (190, 191). From basic cell biology to the production of advanced industrial materials, *Caulobacter* remains at the forefront of bacterial research as we continue to learn more about this fascinating organism.

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