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SPATIAL AND TEMPORAL CONTROL OF DIFFERENTIATION AND CELL CYCLE PROGRESSION IN *CAULOBACTER CRESCENTUS*

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■ **Abstract** The dimorphic and intrinsically asymmetric bacterium *Caulobacter crescentus* has become an important model organism to study the bacterial cell cycle, cell polarity, and polar differentiation. A multifaceted regulatory network orchestrates the precise coordination between the development of polar organelles and the cell cycle. One master response regulator, CtrA, directly controls the initiation of chromosome replication as well as several aspects of polar morphogenesis and cell division. CtrA activity is temporally and spatially regulated by multiple partially redundant control mechanisms, such as transcription, phosphorylation, and targeted proteolysis. A multicomponent signal transduction network upstream CtrA, containing histidine kinases CckA, PleC, DivJ, and DivL and the essential response regulator DivK, contributes to the control of CtrA activity in response to cell cycle and developmental cues. An intriguing feature of this signaling network is the dynamic cell cycle–dependent polar localization of its components, which is believed to have a novel regulatory function.

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INTRODUCTION

The Aim and Scope of This Review

Our goal is to discuss the current knowledge and views on the signaling mechanisms that communicate spatial and temporal information between two major built-in cellular programs, the cell cycle and the development of polar organelles, in the dimorphic bacterium *Caulobacter crescentus*. Key players in these complex processes are members of the superfamily of so-called two-component signal transduction proteins. We summarize extensive experimental work from several *Caulobacter* laboratories that has contributed to our understanding of the multicomponent regulatory network and of its multifaceted control mechanisms that govern the precise coordination between morphological differentiation and cell cycle progression in *Caulobacter*. The control mechanisms specific to polar organelle biogenesis, cell division, and chromosome replication in *Caulobacter* are not discussed here but have been comprehensively reviewed elsewhere (1, 15, 32, 41).

Caulobacter crescentus—A Dimorphic Polarized Bacterium

Members of the genus *Caulobacter* are dimorphic, stalked (prosthecate) bacteria and inhabit almost all water bodies on Earth, where they play an important role in global carbon cycling by mineralization of dissolved organic material (47). One of the most spectacular features of these bacteria is dimorphism. In *Caulobacter* dimorphism is maintained by obligate asymmetric cell division at each reproductive cycle, giving rise to two genetically identical but morphologically different daughter cells: a sessile cell equipped with an adhesive stalk and a motile flagellated swarmer cell (9) (Figure 1). The two daughter cells also inherit a different developmental program. The progeny stalked cell starts a new replicative cycle immediately after cell division. In contrast, the progeny swarmer cell has first an obligate motile life phase, during which the DNA replication and cell division programs are inhibited. After this motile period the swarmer cell undergoes cellular differentiation, which involves ejection of the flagellum, retraction of the pili, and generation of a stalk at the pole previously occupied by the flagellum and

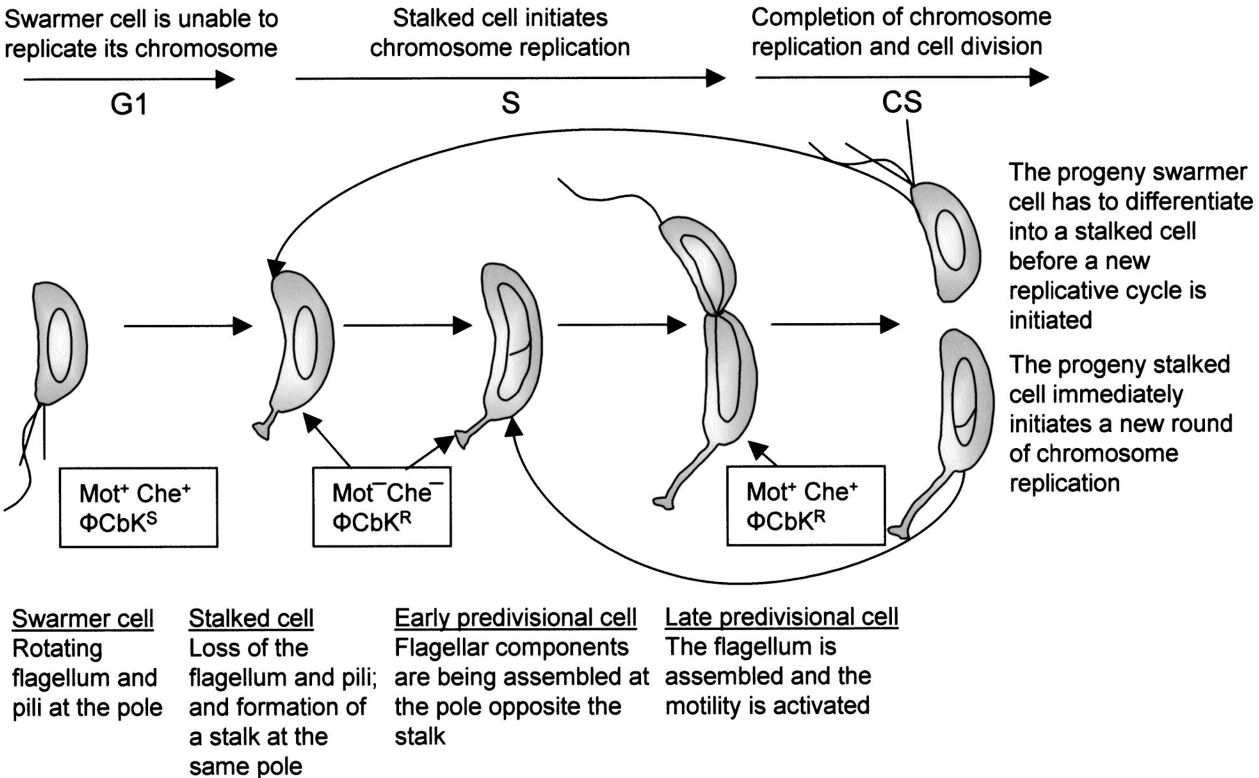

Figure 1 Schematic cell cycle of *C. crescentus*. Phenotypes associated with the presence or absence of polar organelles are indicated: Mot, motility; Che, chemotaxis; ΦCbK^S and ΦCbK^R, sensitivity or resistance to polar bacteriophage ΦCbK, respectively, which indicates the presence or absence of polar pili because they provide the receptors for phage ΦCbK. The stages of cell cycle progression (G1, lag phase before DNA replication; S, a period of new DNA synthesis; and CS, cell separation) are indicated. Open and divided circles inside a cell depict the nonreplicating and replicating chromosomes, respectively.

pili (Figure 1). Coincidentally with these developmental events the new stalked cell becomes reproduction competent and initiates a new round of DNA replication. This motile G1 phase in the swarmer cell cycle presumably gives the cell an opportunity to search for nutrients in new areas away from the sessile stalked mother cell. Thereby, the population is kept disperse to minimize competition for resources. This kind of dimorphism is believed to have evolved to cope with life in dilute nutrient-poor environments (47). The stalk in Caulobacteria is a cellular appendix, which consists mostly of cell envelope material and cytoplasm with no DNA or ribosomes (9). The stalk mediates attachment to surfaces through a holdfast organelle at its tip. It also constitutes an important means of adaptation to constant famine. Its elongation can considerably increase the total cell surface area, thereby increasing nutrient uptake (27). This is illustrated by phosphate starvation, which results in elongation of the stalk several times the length of the cell body (18, 58).

Why Study *Caulobacter*?

These ubiquitous, stalked dimorphic bacteria are exciting microorganisms to study from the evolutionary or ecological point of view. However, the lion's share of research involving *Caulobacter* has been dedicated to unraveling the mechanisms that control polar morphogenesis and cell cycle progression. *C. crescentus* has now taken a place beside *Escherichia coli* and *Bacillus subtilis* as an important model system to study the genetic and regulatory network that controls the bacterial cell cycle in both temporal and spatial dimensions. Owing to its built-in differentiation program that occurs invariably at each cell cycle, cell cycle progression can easily be followed in the laboratory by monitoring simple phenotypic traits, such as motility, chemotaxis, or susceptibility to a pilus-specific bacteriophage, which are characteristic of each cell cycle phase (Figure 1). Another unique strength of the *Caulobacter* system is the ease to obtain synchronized cell populations with a density gradient centrifugation that separates swarmer cells from stalked cells (16). This allows examination of changes in morphology, mRNA levels, protein levels, protein modification, and protein localization during the course of the cell cycle. *Caulobacter* is easily amenable to genetic, biochemical, and cell biological dissection. Its genome has been completely sequenced and annotated (43). It encodes approximately 3700 predicted genes that can be monitored for their expression in a systematic fashion with DNA microarrays (28, 35, 36).

Because of the high tractability of the *Caulobacter* system, insights have been gained into the cellular organization of prokaryotes and the mechanisms used by them to control temporal and spatial processes, such as those involved in cell cycle control. Another unique aspect of *Caulobacter* is its inherent cell polarity. *Caulobacter* offers an opportunity to unravel, in its most basic and primitive form, the mechanisms governing cell polarity—one of the most basic principles in biology.

THE ORDERED EVENTS OF POLAR MORPHOGENESIS AND CELL CYCLE ARE MIRRORED IN THE GLOBAL PROFILES OF GENE AND PROTEIN EXPRESSION

The small size of the *Caulobacter* genome and the ease of obtaining synchronized cell populations have opened the door to genome- and proteome-wide studies with the long-term goal to attain a global and integrated picture of the differentiation and cell cycle processes. The first attempt in this direction was to address how the overall transcription profile changes during the course of the cell cycle. A DNA microarray analysis of 90% of all predicted genes showed that 19% significantly change their expression at the mRNA level as a function of the cell cycle (36). Even though the composition of the set of cell cycle–regulated genes depends on the statistical approach used, it contained all previously characterized cell cycle–regulated genes and their expression patterns were in good agreement with what had been determined by Northern blot analysis or *lacZ* fusions (36). This global analysis revealed an overall temporal correlation between the time of gene expression and the time when the corresponding gene product is needed. Genes involved in the initiation of chromosome replication, DNA methylation, chromosome segregation, cell division, and membrane and peptidoglycan synthesis were expressed in accordance with the time of their expected function (36). Similarly, genes encoding proteins participating in the assembly of polar organelles, such as the flagellum and pili, were expressed in regulatory cascades, reflecting the order of assembly of their gene products (36). Thus, transcriptional control clearly plays a crucial role in the temporal regulation of polar morphogenesis and the cell cycle. Another interesting observation that came from this global gene expression study was that the expression of ribosomal genes and genes related to general metabolism and oxidative respiration was upregulated in S-phase, indicating that a large part of the general metabolism and other cellular housekeeping activities may be under cell cycle control (36).

To complement the genome-wide gene expression data, Grunenfelder et al. (19) used ^{35}S-methionine cell labeling and two-dimensional gel electrophoresis coupled to mass spectrometry to examine protein expression of synchronized cell populations during the course of the cell cycle. In good agreement with the microarray data a large portion of detected proteins (15%), including many metabolic proteins, were differentially synthesized during the cell cycle. Because the actual amount of a given protein at a certain time point during the cell cycle is dependent not only on its expression level but also on its stability, the study also addressed the question of protein stability at a proteome-wide level by pulse-chase ^{35}S-methionine cell labeling (19). An important finding was that a strong correlation could be established between a protein's instability and its differential synthesis during the cell cycle. In other words, proteins with a cell cycle–regulated expression were more likely to be unstable relative to the length of the cell cycle than proteins constitutively expressed during the cell cycle. This indicates that rapid (and targeted) degradation of proteins is an important mechanism to generate periodic changes

in their abundance during the cell cycle, suggesting a global role of proteolysis in the regulation of the bacterial cell cycle.

These two studies showed that whole-genome approaches are of great value in identifying global patterns of temporal gene and protein regulation. They also identified candidate genes for a role in cell cycle control. For instance, the DNA microarray study showed that 25 genes encoding putative signal transduction proteins were expressed in a cell cycle–dependent manner (36); of these, 13 were involved in different aspects of polar morphogenesis or cell cycle regulation. Studying the remaining 12 genes individually is likely to uncover several missing links of known signal transduction pathways.

GLOBAL REGULATION BY A MASTER RESPONSE REGULATOR

Transcriptional control of gene expression plays a critical role in determining the temporal occurrence of events during the cell cycle. What are the factors involved in transcriptional regulation? Because the polar morphogenetic events in *Caulobacter* invariably happen in coordination with cell cycle progression, it was originally proposed that the cell cycle acts as a biological clock that provides cues for the timing of events involved in morphological differentiation (25). One important early observation was that chromosome replication was required for flagellum formation (61), suggesting that a common regulator controls both the transcription of early flagellar genes and the initiation of DNA replication. Owing to the latter activity, this gene was expected to be essential for viability. On the basis of this logic, a genetic screen was designed to isolate temperature-sensitive (ts) mutants that were defective in the regulation of an early flagellar gene, *fliF*, at 28°C (which is the normal growth temperature used in laboratory) and had a lethality defect at 37°C (50). Pursuing this screen and another one with a similar logic yielded the identification of two essential signaling proteins, the CtrA response regulator and the CckA histidine kinase (29, 50). CtrA and CckA belong to the superfamily of two-component signal transduction proteins, which play a major role in signal transduction in bacteria (70). In the two-component paradigm, upon receiving a signal on its sensor domain, the histidine kinase autophosphorylates on a conserved histidine residue of its transmitter domain (75). Signal transduction is achieved by the transfer of the phosphoryl group onto a conserved aspartate residue in the receiver domain of the cognate response regulator. Phosphorylation of the response regulator results in execution of the output response, which often is transcriptional activation or repression of target genes (70). A variation of the two-component system is the multicomponent phosphorelay signal transduction system, in which a receiver domain resembling those found in response regulators and a histidine phosphotransferase domain participate in a phosphorelay that culminates in the phosphorylation of the response regulator that mediates the output response. In these multicomponent phosphorelays the phosphoryl group is invariably transferred in the direction histidine → aspartate → histidine → aspartate

between domains that can be covalently linked or not. Each additional component is potentially subject to additional control (24).

CtrA—An Essential Transcriptional Regulator that Controls Both Polar Morphogenesis and Cell Cycle Processes

CtrA is a response regulator with a conventional structure consisting of a conserved N-terminal receiver domain and a C-terminal DNA binding output domain (50). Phosphorylation at the conserved aspartate residue (Asp51) of the receiver domain enhances the binding activity of CtrA for its target DNA sequences (52, 62). Accordingly, phosphorylation of CtrA (CtrA ~ P) is essential for its activity and therefore for cell viability (50). Interestingly, already before the discovery of CtrA, a conserved 9-mer sequence motif had been detected in the promoter regions of many cell cycle–regulated genes, including several of the early flagellar genes (68, 78), the essential DNA methyltransferase encoding gene *ccrM* (69), and the *hemE* P_s promoter that resides within the chromosomal origin of replication (39, 40). DNA footprinting and genome-wide location experiments have shown that CtrA ~ P binds to this conserved 9-mer sequence motif (5, 35, 42, 46, 51). CtrA controls both polar morphogenesis and essential cell cycle processes. For instance, expression of *ftsZ* encoding the essential cell division protein FtsZ is directly controlled by CtrA ~ P (34, 35). Transcriptional control of *ftsZ* is an important mechanism by which to control the abundance of FtsZ in the cell, thereby regulating the initiation of cell division (34, 48, 49). CtrA also controls the expression of *ccrM*, a gene encoding an essential DNA methyltransferase that is involved in cell cycle control (35, 53, 67, 79). Similarly, CtrA controls the expression of many genes involved in flagellar synthesis, pili assembly, and chemotaxis (5, 33, 37, 42, 46, 63). Comparative expression microarray experiments performed on a wild-type *Caulobacter* strain and a derivative strain harboring a *ctrA* ts loss-of-function allele indicated that a third of the cell cycle–regulated genes are directly or indirectly under the control of CtrA (36). The direct gene targets of CtrA were later determined in a genome-wide location study, where fragments of genomic DNA were recovered by immunoprecipitation using anti-CtrA antibodies after in vivo binding and subsequent cross-linking of CtrA to its target promoters (35). This study showed that CtrA directly bound as many as 55 promoters, controlling 95 genes (some of which were organized in operons). In addition to its role as a global transcriptional regulator, CtrA ~ P represses the initiation of chromosome replication by directly binding to five sites within the chromosomal origin of replication (51, 62). These CtrA binding sites overlap an essential DnaA box and a promoter in the origin of replication, both of which are essential for initiation of chromosome replication (39).

By controlling polar morphogenesis on the one hand and the initiation of chromosome replication and cell division on the other, CtrA orchestrates the coordinated progression of these distinct events. Thus, the control of CtrA activity, which determines when these cell cycle events occur, is critical for the cell.

Multiple Spatial and Temporal Mechanisms Regulate CtrA Activity

CtrA activity is controlled temporally at three levels: transcription, proteolysis, and phosphorylation (Figure 2). The transcription of *ctrA* is cell cycle regulated, with a peak expression in the predivisional cell stage (14, 36, 50) (Figure 2). Expression of *ctrA* is under the control of two promoters, P1 and P2, that are active at different times during the predivisional stage and are directly regulated by CtrA itself (14). The weaker P1 promoter is active in the early predivisional cell and is repressed by CtrA, whereas the stronger P2 promoter is activated later during the predivisional stage and is positively regulated by CtrA. Thus, the absence of active CtrA in the stalked cells due to proteolysis and dephosphorylation (as explained below) presumably triggers the expression of CtrA from the weaker P1 promoter in the early predivisional cells. Accumulation of CtrA (and subsequent phosphorylation of it) causes the repression of the P1 promoter and the activation of the stronger P2 promoter, resulting in elevated levels of the CtrA protein in the late predivisional cell (14).

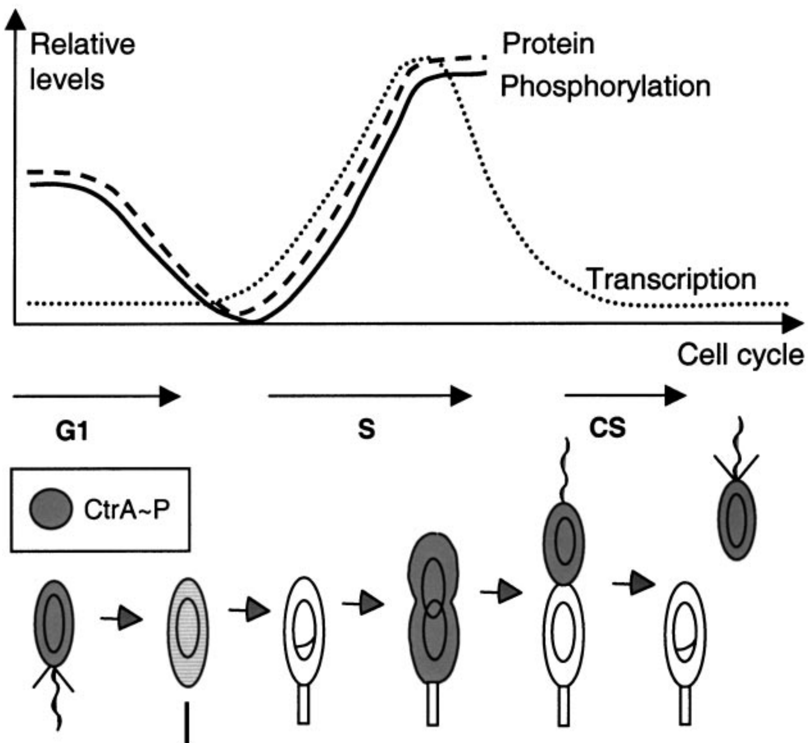

Figure 2 Schematic representation of the regulatory mechanisms that modulate the level of CtrA ∼ P (in gray) during the cell cycle.

As mentioned above, to be active, CtrA has to be phosphorylated (28, 50). In vivo phosphorylation experiments on synchronized cell populations showed that the levels of CtrA ~ P change dynamically during the course of cell cycle (as schematically illustrated in Figure 2) (13, 28). CtrA is present and phosphorylated in swarmer cells. The level of CtrA ~ P drops during the swarmer-to-stalked (G1-S) cell transition. When CtrA is resynthesized by transcriptional activation in early predivisional cells, the CtrA ~ P level rises to reach the highest level in predivisional cells (Figure 2). Because CtrA ~ P represses initiation of DNA replication by directly binding to the origin of replication (51), the disappearance of CtrA ~ P during the G1-S cell transition relieves repression and thereby allows initiation of DNA replication to occur. Two redundant mechanisms are responsible for removing CtrA ~ P from the cells at the G1-S cell transition. One mechanism is the targeted proteolysis of CtrA by the ClpXP protease complex (13, 31). CtrA is specifically degraded during the G1-S cell transition (Figure 2). However, when a proteolysis-resistant CtrA derivative is expressed in the cells, polar morphogenesis and the cell cycle still proceed normally (13). This is because, even though the protein persists during the G1-S cell transition and thereafter, it is dephosphorylated and therefore inactivated at the right time, allowing normal cell cycle progression (13). Thus, CtrA phosphorylation is also cell cycle regulated (Figure 2). A constitutively active mutant of CtrA (CtrAD51E) that does not require phosphorylation to be active (and therefore cannot be dephosphorylated) is also harmless to the cells because the protein is degraded at the G1-S cell transition (13). Thus, dephosphorylation of CtrA ~ P and ClpXP-mediated proteolysis constitute two redundant mechanisms that ensure clearance of CtrA activity during the G1-S cell transition. Consistently, the presence of a CtrA mutant that is both constitutively active and resistant to ClpXP-mediated proteolysis confers a dominant G1 cell cycle arrest by preventing initiation of DNA replication to occur (13).

CtrA ~ P is also degraded, and presumably dephosphorylated, in the stalked compartment of the late predivisional cell, whereas it remains stable in the swarmer compartment (13) (Figure 2). Upon cell division the asymmetry in CtrA ~ P levels in the late predivisional cell generates a swarmer daughter cell with CtrA activity and a stalked daughter cell without CtrA activity. This segregation in CtrA activity results in a different competence in DNA replication and in a different program of gene expression between the two daughter cells. Thus, CtrA ~ P acts as a cell fate determinant.

An interesting and important question is, What are the cell cycle cues that control the timing of CtrA ~ P degradation and dephosphorylation during the G1-S cell transition and in the stalked compartment of the late predivisional cell? The levels of ClpX and ClpP proteins are constant throughout the cell cycle (31), suggesting that a hitherto unidentified regulatory mechanism controls the onset of CtrA degradation by the ClpXP protease complex. A recent study showed that the first 56 residues of the N-terminal receiver domain together with the last 15 residues of the C-terminal domain contain the information necessary for the regulated proteolysis of CtrA (57). Little is known about the mechanism of CtrA ~ P

dephosphorylation. A significant observation that has been underappreciated is that the half-life of CtrA ~ P as determined in vivo (under 5 min) is considerably shorter than its half-life as measured in vitro with purified CtrA ~ P (30 min) (13, 77), indicating that dephosphorylation of CtrA ~ P is actively carried out by proteins that remain to be identified.

How Does CtrA Control Gene Expression Throughout the Cell Cycle?

CtrA does not exert its regulation only at one specific cell cycle stage. Instead, CtrA-dependent events occur throughout the cell cycle. This is best illustrated by the observation that the 55 promoters that are directly controlled by CtrA ~ P show peak expressions at different times during the cell cycle (35, 36). How can a single transcriptional regulator have such a global impact throughout the cell cycle? One of the reasons lies in the ability of CtrA ~ P to either activate or repress gene transcription. Consequently, genes repressed by CtrA ~ P are typically expressed during the G1-S cell transition when CtrA activity is cleared from the cells, whereas genes activated by CtrA ~ P are maximally expressed in the predivisional cells when CtrA is present and phosphorylated at the highest level (35) (Figure 2).

Another mechanism governing temporal regulation of gene expression relies on the binding affinity of CtrA ~ P for the promoter region and the regulation of CtrA ~ P levels during the cell cycle. The levels of CtrA ~ P are significantly higher in predivisional cells than in swarmer cells (13, 28) (Figure 2), and this difference may be sufficient to differentially regulate the temporal expression of different genes. In this context, low levels of CtrA ~ P in swarmer cells bind only to high-affinity CtrA binding sequences, such as those in the chromosomal origin of replication (35, 51, 62). Later, the combined action of dephosphorylation and degradation of CtrA ~ P during the G1-S cell transition results in initiation of DNA replication. In early predivisional cells CtrA is resynthesized and immediately phosphorylated to ensure that no extra round of initiation of DNA replication occurs. As CtrA ~ P levels rise in predivisional cells, CtrA ~ P binds and activates gene promoters in the reverse order of their binding affinity for CtrA ~ P. For example, early flagellar genes and the *ccrM* gene are expressed in the early and late predivisional cell stages, respectively (52). Accordingly, a 10- to 20-fold difference in CtrA ~ P binding affinities of the *ccrM* and flagellar *fliQ* promoters contributes to the sequential transcription of these genes in the predivisional stage of the cell cycle (52). Thus, the careful control of CtrA phosphorylation levels during the entire course of the cell cycle may play a major role in orchestrating the orderly sequence of cell cycle events.

It is likely that other mechanisms and factors (such as accessory regulatory proteins) help fine-tune the precise timing of CtrA-regulated gene expression. For example, changes in the methylation state of a promoter can alter the timing of gene transcription during the cell cycle (53, 69).

THREE SIGNAL TRANSDUCTION PATHWAYS UPSTREAM CtrA

Given its importance, the cellular level of CtrA ~ P must be precisely controlled throughout the cell cycle. At least three signal transduction pathways have been proposed to control CtrA phosphorylation (Figure 3): the CckA pathway, the DivL pathway, and the PleC-DivJ-DivK multicomponent pathway. This multipathway signaling network, which ultimately leads to the precise and dynamic control of CtrA activity during the cell cycle, has not yet been completely reconstituted. Several components of the network are clearly missing and some functional aspects are not clearly understood. However, many important pieces of the puzzle are known and can be linked together into a provisional picture of how signals are processed and transduced to the master regulator, CtrA.

The CckA Pathway

One genetic pathway required for CtrA phosphorylation contains the CckA histidine kinase, which is essential for viability (28, 29). Several lines of genetic evidence indicate that CckA exerts its essential function mainly through CtrA regulation. A strain harboring a ts loss-of-function allele of *cckA* displays morphological and cell cycle–related defects similar to those of a ts loss-of-function allele of *ctrA* (29). This is further supported by a comparative microarray analysis of *ctrA* and *cckA* ts mutants that showed that their mutations affect the mRNA levels of an almost identical set of cell cycle–regulated genes at the restrictive temperature (28). Most importantly, a CtrA derivative, CtrAD51E, that does not require phosphorylation to be active when overexpressed supports viability in the absence

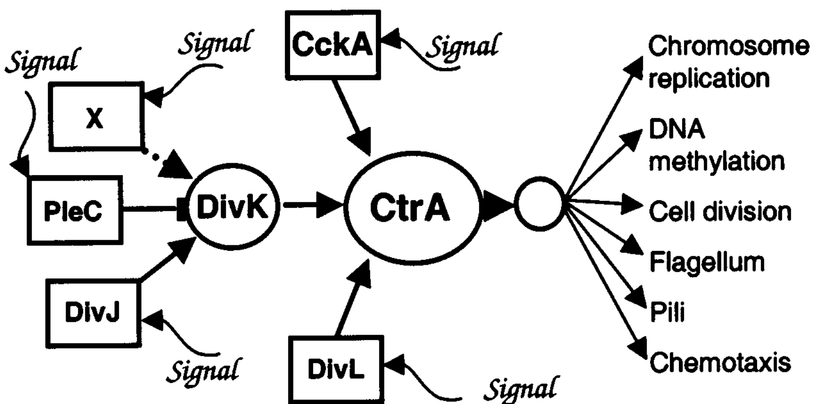

Figure 3 A model of the signal transduction network controlling the activity of the master response regulator, CtrA. Response regulators and histidine kinases are shown as circles and boxes, respectively.

of CckA, whereas overexpression of wild-type CtrA does not (28). This indicates that CckA is essential for viability because it is required for CtrA phosphorylation. Consistently, the level of CtrA ~ P is radically reduced upon partial loss of CckA function (29). The cell cycle–dependent pattern of CckA phosphorylation, which presumably reflects its activity, correlates with that of CtrA (28), suggesting that CckA may control CtrA phosphorylation throughout the cell cycle.

CckA is a hybrid histidine kinase. In addition to the conserved sequences of a prototypical sensor histidine kinase, CckA has a response regulator receiver domain at its C terminus (29). According to the multicomponent signal transduction paradigm (24), CckA's hybrid architecture predicts a phosphorelay with an unidentified histidine phosphotransferase between CckA and CtrA. Therefore, in vitro reconstitution of the CckA-CtrA pathway may have to await the identification of this putative histidine phosphotransferase.

DivL: A Sensory Kinase with a Unique Structure

DivL is another sensory kinase that has been genetically linked to CtrA. Strains carrying conditional alleles of *divL* exhibit phenotypes, including extreme cell filamentation at the restrictive temperature (77), similar to those of *ctrA* ts strains. The most compelling evidence of a genetic interaction between DivL and CtrA is that a *ctrA* conditional allele (*sokA*) suppresses the cold-sensitive (cs) phenotype of a *divL* allele, arguing that DivL functions genetically upstream CtrA (77). Although there is no in vivo phosphorylation data available yet, a purified preparation of a soluble truncated version of DivL is able to phosphorylate CtrA in vitro (77). Intriguingly, DivL has all the conserved regions (H-, N-, D-, and G-box sequences) present in the bacterial histidine kinase superfamily except that a tyrosine residue (Tyr550) is autophosphorylated in place of a histidine residue (77). This tyrosine residue is critical for the essential function of DivL in vivo (77). It will be interesting to determine how the change of a catalytic residue from histidine to tyrosine affects the signaling properties of a bacterial sensory kinase.

The PleC-DivJ-DivK Multicomponent Signal Transduction Pathway

A third signaling pathway containing the essential response regulator, DivK, and the two nonessential histidine kinases, PleC and DivJ, acts genetically upstream CtrA to control its activity. This was demonstrated using the conditional *ctrA* allele (*sokA*) shown to suppress the cs phenotype of a *divL* mutation (77). Similarly, the *sokA* allele suppresses the lethality of a *divK* null mutation, the cs cell division phenotype of a *divJ* mutant, and the nonmotile and stalkless phenotypes of a *pleC* mutant (59, 76). The exact mechanism by which the *sokA* allele suppresses the phenotypes associated with mutations in *divL*, *divK*, *divJ*, and *pleC* is not known. However, the *sokA* suppression analysis establishes a genetic link between these upstream signaling proteins and CtrA. The *divJ* and *divK* genes were initially identified from a clever pseudoreversion genetic screen designed to isolate mutations

that suppress the ts nonmotile phenotype of a *pleC* mutant at 37°C and that confer a cell division defect at 24°C (65). The rationale for the screen was based on an early observation that cells blocked in early stages of the cell cycle assemble nonmotile flagella and do not form stalks (25), indicating that one or several cell division regulators participate directly in the regulation of polar morphogenesis. Mutant alleles of *divJ* and *divK* (and *divL*) were recovered as suppressors of *pleC*, indicating that *pleC*, *divJ*, and *divK* interact genetically to regulate both cell division and motility (65). This was in fact the first direct genetic evidence that the polar morphogenesis and cell cycle pathways were interconnected.

PleC AND DivJ HISTIDINE KINASES SIGNAL DIFFERENT CELL CYCLE EVENTS VIA THE DivK RESPONSE REGULATOR PleC is a prototypical membrane-bound histidine kinase that controls the differentiation of the swarmer pole into a stalked pole (64, 73). PleC function is needed to activate motility and chemotaxis after completion of the flagellum assembly in the predivisional cell (10, 64, 65). *pleC* mutants are defective in all consecutive events in the swarmer pole differentiation after the activation of motility: (*a*) the formation of polar pili, (*b*) the loss of the flagellum and pili during the G1-S cell transition, and (*c*) the subsequent formation of the stalk (Figure 4). Consequently, the predivisional cells of *pleC* mutants appear morphologically symmetrical with nonmotile flagella at both poles and divide into seemingly similar progeny cells (64, 73). Thus, PleC plays a central role in timing morphogenetic processes with the cell cycle.

DivJ is also a membrane-bound histidine kinase (44), whose function is needed for both controlling the placement and length of the stalk and initiating cell division; *divJ* mutants are elongated and have long stalks that are often misplaced (44, 65, 74).

DivK is a single-domain response regulator protein with a function essential for cell viability (21). In contrast to the prototypical response regulator, which contains a receiver domain and an output domain, DivK consists solely of the receiver domain (20, 21). Response regulators lacking the output domain function to either mediate the output response directly via protein-protein interactions (like the CheY chemotaxis regulator) or participate in a multicomponent phosphorelay as a phosphointermediate between a histidine kinase and a histidine phosphotransferase (like the Spo0F sporulation regulator of *B. subtilis*) (70). DivK has pleiotropic functions in the regulation of both morphogenesis and cell cycle processes. Conditional mutations in *divK* cause abnormal stalk formation, severe cell filamentation, block in initiation of chromosome replication, and ultimately cell death at the restrictive temperature (21, 26, 65). DivK function is genetically linked to PleC function in regulating motility because point mutations in *divK* suppress the motility defect caused by a *pleC* mutation (65). DivK has also been proposed as a downstream target of the DivJ histidine kinase in the regulation of cell division (21, 76), suggesting that PleC and DivJ are the cognate histidine kinases of DivK. This idea is supported by the observation that purified preparations of DivJ and PleC kinase domains, which share extensive sequence similarity, can

efficiently phosphorylate DivK and dephosphorylate DivK ∼ P in vitro (21, 44, 73). Histidine kinases can be involved not only in the phosphorylation but also in the dephosphorylation of their cognate response regulators. These bifunctional histidine kinases are often observed in signal transduction pathways that need to be shut down quickly (70). How does a shared response regulator (DivK) differentially mediate the responses of two histidine kinases (DivJ and PleC) that control distinct morphogenetic and cell cycle events? One clue originates from the in vivo phosphorylation data showing that the levels of DivK ∼ P are decreased in a *divJ* mutant but increased in a *pleC* mutant relative to wild type (74). Thus, PleC and DivJ have opposite effects in the control of DivK phosphorylation in vivo (Figure 3). The kinase activity of DivJ contributes to the production of DivK ∼ P, while PleC functions, directly or indirectly, to dephosphorylate DivK ∼ P. Although genetic and biochemical data strongly support the idea that PleC and DivJ are cognate kinases of DivK, it is also possible that PleC, instead of acting directly on DivK ∼ P as a phosphatase, inhibits the phosphorylation of DivK. In a Δ*divJ* single mutant and Δ*divJ pleC*::Tn5 double mutant, the level of DivK ∼ P is considerably reduced but not abolished (74), indicating that at least another kinase, yet to be identified, contributes to the formation of DivK ∼ P (Figure 3). This is consistent with the fact that DivK is essential for viability, whereas PleC and DivJ are not (21, 64, 73, 74).

To add another interesting twist to the story, PleC is also genetically linked to the response regulator PleD, which together with PleC is involved in the differentiation of the swarmer pole (22, 64). PleD is required for the cell cycle–dependent proteolytic degradation of the FliF flagellar anchor protein, which results in the loss of motility and ejection of the flagellum during the G1-S cell transition (2). Thus, two consecutive events of the swarmer pole differentiation are controlled by PleC via signaling to two different response regulators. First, PleC-DivK signaling results in the gain of motility in the predivisional cells, and subsequently, PleC-PleD signaling causes the loss of motility during the G1-S cell transition of the next swarmer cell cycle.

HOW DOES DivK REGULATE CtrA ACTIVITY? As mentioned before, the essential function of DivK lies in the regulation of CtrA because the *ctrA* allele *sokA* suppresses the lethality of a *divK* null mutation (76). How this is accomplished has not yet been conclusively determined. Two nonexclusive models have been proposed. In the first model DivK controls the level of CtrA ∼ P in a phosphorelay with an unknown histidine phosphotransferase between DivK and CtrA (76). In the second model DivK is involved in the degradation of CtrA during the G1-S cell transition (26). A synchronized population of the cs *divK* mutant *divK341* (21) arrests in G1 phase at the restrictive temperature; in these cells initiation of DNA replication is blocked, genes involved in DNA replication are not induced, and CtrA and the chemoreceptor McpA, which are both normally degraded by the ClpXP protease complex during the G1-S cell transition, remain stable (26). These results led to the suggestion that DivK may be involved in the temporal control of ClpXP activity (26). Expression of a proteolysis-resistant CtrA mutant protein throughout the cell

cycle does not affect cell cycle progression because the protein is still inactivated by dephosphorylation during the G1-S cell transition (13). Therefore, the retention of CtrA alone cannot account for the phenotypes observed in *divK341* cells at the restrictive temperature, suggesting that DivK may be involved in the control of both proteolysis and phosphorylation of CtrA. Further experiments are necessary to ascertain the mechanism by which DivK regulates CtrA activity.

A MULTIPATHWAY NETWORK THAT REGULATES CtrA ACTIVITY Figure 3 illustrates a simplistic view of the regulatory network that governs cellular differentiation and cell cycle progression in *Caulobacter*. In this proposed model the global transcriptional regulator CtrA occupies the central position. Three upstream pathways, the CckA pathway, the DivL pathway, and the PleC-DivJ-DivK multicomponent pathway, contribute to the control of the CtrA ∼ P level. DivK is a central player in the network because it integrates multiple morphogenetic and cell cycle cues. DivK regulates the level of CtrA ∼ P, and therefore CtrA activity, by signaling into a putative phosphorelay that ultimately modulates the phosphorylation level of CtrA and/or by controlling the degradation of CtrA.

DYNAMIC SUBCELLULAR LOCALIZATION OF THE SIGNALING PROTEINS—HOW AND WHY?

The complex cellular processes of an intricate signal transduction network interconnect polar morphogenesis and cell cycle progression in *Caulobacter*. Components of this network are subjected to stringent control on multiple levels. One of the most fascinating facets of this network is that most of its components have a distinct subcellular localization pattern. Protein localization often is dynamic, alternating between dispersed distribution and discrete localization at a cell pole. The time of localization of a signaling protein at the cell pole often coincides with the time window when the protein is most active or when its function is needed. Therefore, localization of signal transduction proteins to specific cellular locations (such as the poles) may be a mechanism to spatially regulate the interplay between components of the cell cycle regulatory network, and thereby mediate the precise coordination between cell cycle events. Thus, spatial cues may play an important role in the control of cell cycle progression in *Caulobacter*. It is noteworthy that other components of the cell, including the chromosomal origins of replication, proteins involved in chromosome segregation, and flagellar and pili structural proteins as well as components of the chemotactic apparatus, also localize at specific cell poles in a cell cycle–regulated fashion (32, 38, 60).

Dynamic Cell Cycle–Dependent Localization of the CckA Histidine Kinase

The membrane-bound CckA histidine kinase dynamically localizes at a cell pole in a cell cycle–dependent manner and alternates from dispersed distribution around

the membrane at early stages of the cell cycle to localization predominantly at the cell pole opposite the stalk in the predivisional cell (29). Polar localization of CckA correlates with its peak phosphorylation, which coincides with the timing of CtrA synthesis and activation, suggesting that CckA polar localization has a regulatory function (28).

Dynamic Localization of the Components of the PleC-DivJ-DivK Pathway

As described before, genetic and biochemical evidence indicates that at least two histidine kinases, PleC and DivJ, signal different cell cycle cues through the DivK response regulator (21, 65, 74). Thus, DivK, and therefore the regulation of its activity, play a pivotal role in coordinating morphogenetic and cell cycle events. The level of DivK ∼ P is significantly lower in swarmer cells than it is in later cell cycle stages where it remains fairly constant (30). However, the most distinctive regulation of DivK during the cell cycle is its dynamic spatial distribution within the cell (30) (Figure 4). DivK is dispersed in the cytoplasm of swarmer cells. As the swarmer cells differentiate into stalked cells, DivK localizes at the pole where a stalk develops (stalked pole). Soon after, in early predivisional cells, some DivK proteins migrate to the pole opposite the stalk (new swarmer pole) to form a second focus of localization (30). DivK remains bipolarly localized until cell division is completed, at which time DivK is released from the new swarmer pole but remains anchored at the base of the stalk. Thus, cell division results in the generation of two progeny cells with different spatial subcellular distribution of DivK: a swarmer cell with DivK evenly distributed throughout the cytoplasm and a stalked cell with DivK polarly localized (30).

The PleC and DivJ histidine kinases, which control antagonistically DivK phosphorylation (74), are also involved in the temporal and spatial regulation of DivK localization (30). DivJ is required for DivK localization at the poles, as demonstrated by the inability of DivK to localize in *divJ* mutants where DivK is uniformly distributed in the cytoplasm of all cell types (30). Conversely, DivK is perfectly able to polarly localize at both poles in *pleC* mutants but fails to delocalize from either pole even after completion of cell division, generating two daughter cells with identical polar localization of DivK (30). This result indicates that PleC is involved in the pole-specific release of DivK at the end of cell cycle.

Interestingly, PleC and DivJ are themselves localized at a specific cell pole at specific stages of the cell cycle (74). Consistent with its role in regulating the growth and positioning of the stalk, DivJ is mainly found localized at the base of the stalk in all stalked cell types (59, 74) (Figure 4). PleC localization is dynamic during the cell cycle (74) (Figure 4). It is localized at the flagellated pole of swarmer cells. During the G1-S transition PleC is released from the pole and redistributed throughout the cytoplasmic membrane to later relocate itself to the pole opposite the stalk in predivisional cells. Thus, PleC localizes at the pole where it governs differentiation events (Figure 4). Both DivJ and PleC are polarly localized where

and when their functions are needed, suggesting a regulatory function for polar localization. Another interesting point is that PleC and DivJ colocalize with DivK simultaneously at opposite poles in predivisional cells (Figure 4), suggesting that, in analogy with eukaryotic systems, asymmetric localization of signaling complexes may provide a mechanism to generate and maintain cellular asymmetry in bacteria.

Mechanisms Responsible for Protein Localization

How do bacterial proteins get to a specific cellular location and remain there for a definite amount of time? What determines the temporal and spatial control of localization of these proteins? Clearly, cellular factors must be involved to mediate the anchoring at the pole. Other factors may govern the temporal and spatial regulation for the targeting and maintenance of proteins at a specific pole, and possibly for bringing additional proteins to the signaling complex. Similarly, *cis*-acting sequences within the protein must participate in the interactions critical for the temporally and spatially regulated polar localization. The study of the cyclical migration of signaling proteins in *Caulobacter* is still in its infancy, but exciting results on the (*trans*-acting) cellular factors and the *cis*-acting sequences involved in protein localization have already emerged from recent studies.

***TRANS*-ACTING FACTORS** PleC and DivJ control DivK localization because *divJ* mutants fail to polarly localize DivK, whereas *pleC* mutants are defective in the pole-specific release of DivK at cell separation (30). However, the molecular mechanism by which DivJ and PleC regulate the dynamic localization of DivK during the cell cycle is unknown. A recent study showed that the activity of PleC also controls its own release from the flagellated pole at the G1-S transition (71). This was demonstrated using a catalytically inactive PleC derivative, $PleC_{H610A}$, in which the conserved phosphorylatable histidine residue was replaced with an alanine residue. When present as the only PleC protein, $PleC_{H610A}$ fails to delocalize from the flagellated pole location during the G1-S cell differentiation, generating predivisional cells with bipolar localization of $PleC_{H610A}$ (71). PleC's activity also restricts localization of structural components of the pilus to only one pole, thereby contributing to the cellular asymmetry that generates two morphologically dissimilar progeny cells at cell division (71). PodJ, a coiled-coil-rich protein, provides positional information for PleC localization (12, 23, 72). PleC is found dispersed all around the cytoplasmic membrane of all cell types of *podJ* mutants (23, 72). PodJ's dynamic cell cycle–dependent localization parallels that of PleC (23, 72). It will be interesting to determine whether PodJ controls PleC localization by acting as a scaffold.

In fact, little is known about the mechanisms that target and maintain proteins to discrete cellular locations in bacteria. Two mechanisms for protein localization have been proposed: direct targeting to a cellular location or diffusion and capture by preexisting localized anchors. The first mechanism is illustrated by the polarly

localized outer membrane IcsA protein, which mediates polymerization of the host actin by *Shigella flexneri* (17). Localization of IcsA is accomplished by its direct targeting exclusively to one cell pole, followed by lateral diffusion, which generates a polar gradient of IcsA in the outer membrane (11, 66). The IcsA polar gradient is maintained and sharpened by nonpolarized proteolysis of IcsA in the outer membrane (54). The sporulation protein SpoIVFB uses a different mechanism. It is first inserted uniformly into the cytoplasmic membrane, then it accumulates by diffusion and capture in the outer forespore membrane, which is its final destination (56).

There is little information about the mechanisms of protein localization in *Caulobacter*. The fact that CckA and DivK are stable relative to the length of cell cycle (29, 30) favors a mechanism of diffusion and capture by polar anchors, whereby the existing pools of CckA or DivK molecules move from a dispersed distribution to a polar localization in a cell cycle–dependent fashion. Even if correct, this mechanism raises the inevitable questions of when and how the anchors are themselves laid down at a specific pole. Morphological differences of cell poles from the lateral sides of rod-shaped bacteria may be involved in polar anchor positioning (38). The new pole may have a unique structure or protein or lipid composition from remnants of the last cell division (38). One could also easily imagine that the presence of polar organelles (stalk, flagellum, or pili) that distinguish one pole from the other may provide the necessary spatial information for protein localization. However, recent genetic experiments demonstrated that the localization of DivJ at the stalked pole does not require elongation of a stalk but rather the completion of certain steps of the G1-S differentiation program, such as the gain of motility (59). It suggests that the temporal control of protein localization is mediated by, or in check with, the completion of certain differentiation events.

CIS-ACTING DETERMINANTS What are the *cis*-acting determinants involved in the temporal and/or spatial control of protein localization? A mutagenesis study showed that the sensor domain of DivJ contains all the sequence information for localization at the stalked pole (59). The sensor domain of DivJ contains the predicted transmembrane domain and a cytoplasmic linker, but it lacks the transmitter domain indicating that the catalytic activity of DivJ is not involved in its localization (59). A short sequence of 61 residues (between residues 251 and 312) in the cytoplasmic linker was shown to be critical for polar localization of DivJ (59). A membrane-bound histidine kinase, PilS, has been shown to localize at the poles of *Pseudomonas aeruginosa* (6). Similar to DivJ, it is the sensor domain of PilS that carries all the information necessary and sufficient for polar localization (6), suggesting shared mechanisms for polar localization of membrane-bound histidine kinases. Identification of the specific residues involved in the temporal and spatial regulation of protein localization is critical because their mutation will directly address how polar localization affects protein function and/or regulation. This information is essential to ascertain the regulatory function of polar

localization suggested by the pleiotropic phenotypes of mutant strains with protein localization defects (for example, *divJ* and *podJ* mutants in which DivK and PleC are delocalized, respectively) (30, 72).

FUTURE PERSPECTIVES

Asymmetry and cellular differentiation are inherent features of both the eukaryotic and the prokaryotic worlds. The universal question is, How is genetic information translated into temporal and spatial information? The high tractability of the *Caulobacter* system and the recent advances in genomic and cell imaging technology have generated unique insights into the fundamental mechanisms underlying the coordination between differentiation and cell cycle progression. A complex phosphorelay of two-component signal transduction proteins is at the heart of differentiation and cell cycle control in this organism. Although key players have been identified and characterized, some components of the signal transduction network are still missing. The identification of new cell cycle regulatory genes will help unravel the multipathway regulatory circuitry controlling cell cycle and cell fate in *Caulobacter*. The dissection of these pathways, which are regulated in time and space, will also provide insights into understanding how they are cued to the progression of the cell cycle. One of the most stunning facets of this regulatory network is the dynamic cell cycle–dependent localization of some of its components. Change in subcellular location is a mechanism frequently used by eukaryotic cells to control the activity of regulatory proteins, which often leads to localized signaling complexes that are critical for cell function. In *Caulobacter* the function of protein localization in cell cycle control and the mechanisms whereby these signaling proteins are targeted and released from discrete locations in the cell remain poorly understood. Studying the cyclical migration of signaling proteins will undoubtedly lead to new exciting discoveries. It is also of great interest that components of the *Caulobacter* cell cycle regulatory network are conserved among other α-proteobacteria, including bacterial species with medical or agronomical importance (3, 4, 7, 8). Cell cycle control processes, albeit poorly understood, are known to play an important role in the invasion and colonization process of pathogens and symbionts (3, 45, 55). Thus, studying the function and regulation of homologs of the *Caulobacter* cell cycle regulatory circuitry may provide a basis for understanding not only the biology of important pathogens and symbionts but also the strategies that they use to infect and replicate within their host.

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LITERATURE CITED

1. Aldridge P, Hughes KT. 2002. Regulation of flagellar assembly. *Curr. Opin. Microbiol.* 5:160–65
2. Aldridge P, Jenal U. 1999. Cell cycle-dependent degradation of a flagellar motor component requires a novel-type response regulator. *Mol. Microbiol.* 32:379–91
3. Barnett MJ, Hung DY, Reisenauer A, Shapiro L, Long SR. 2001. A homolog of the CtrA cell cycle regulator is present and essential in *Sinorhizobium meliloti*. *J. Bacteriol.* 183:3204–10
4. Bellefontaine AF, Pierreux CE, Mertens P, Vandenhaute J, Letesson JJ, Bolle XD. 2002. Plasticity of a transcriptional regulation network among alpha-proteobacteria is supported by the identification of CtrA targets in *Brucella abortus*. *Mol. Microbiol.* 43:945–60
5. Boyd CH, Gober JW. 2001. Temporal regulation of genes encoding the flagellar proximal rod in *Caulobacter crescentus*. *J. Bacteriol.* 183:725–35
6. Boyd JM. 2000. Localization of the histidine kinase PilS to the poles of *Pseudomonas aeruginosa* and identification of a localization domain. *Mol. Microbiol.* 36:153–62
7. Brassinga AK, Siam R, Marczynski GT. 2001. Conserved gene cluster at replication origins of the alpha-proteobacteria *Caulobacter crescentus* and *Rickettsia prowazekii*. *J. Bacteriol.* 183:1824–29
8. Brassinga AK, Siam R, McSween W, Winkler H, Wood D, Marczynski GT. 2002. Conserved response regulator CtrA and IHF binding sites in the alpha-proteobacteria *Caulobacter crescentus* and *Rickettsia prowazekii* chromosomal replication origins. *J. Bacteriol.* 184:5789–99
9. Brun YV, Janakiraman RS. 2000. The dimorphic life cycle of *Caulobacter* and stalked bacteria. In *Prokaryotic Development*, ed. YV Brun, LJ Shimkets, pp. 297–317. Washington, DC: ASM Press
10. Burton GJ, Hecht GB, Newton A. 1997. Roles of the histidine protein kinase *pleC* in *Caulobacter crescentus* motility and chemotaxis. *J. Bacteriol.* 179:5849–53
11. Charles M, Perez M, Kobil JH, Goldberg MB. 2001. Polar targeting of *Shigella* virulence factor IcsA in *Enterobacteriacae* and *Vibrio*. *Proc. Natl. Acad. Sci. USA* 98:9871–76
12. Crymes WB Jr, Zhang D, Ely B. 1999. Regulation of *podJ* expression during the *Caulobacter crescentus* cell cycle. *J. Bacteriol.* 181:3967–73
13. Domian IJ, Quon KC, Shapiro L. 1997. Cell type-specific phosphorylation and proteolysis of a transcriptional regulator controls the G1-to-S transition in a bacterial cell cycle. *Cell* 90:415–24
14. Domian IJ, Reisenauer A, Shapiro L. 1999. Feedback control of a master bacterial cell-cycle regulator. *Proc. Natl. Acad. Sci. USA* 96:6648–53
15. England JC, Gober JW. 2001. Cell cycle control of cell morphogenesis in *Caulobacter*. *Curr. Opin. Microbiol.* 4:674–80
16. Evinger M, Agabian N. 1977. Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. *J. Bacteriol.* 132:294–301
17. Goldberg MB, Theriot JA. 1995. *Shigella flexneri* surface protein IcsA is sufficient to direct actin-based motility. *Proc. Natl. Acad. Sci. USA* 92:6572–76
18. Gonin M, Quardokus EM, O'Donnol D, Maddock J, Brun YV. 2000. Regulation of stalk elongation by phosphate in *Caulobacter crescentus*. *J. Bacteriol.* 182:337–47
19. Grunenfelder B, Rummel G, Vohradsky J, Roder D, Langen H, Jenal U. 2001. Proteomic analysis of the bacterial cell cycle. *Proc. Natl. Acad. Sci. USA* 98:4681–86
20. Guillet V, Ohta N, Cabantous S, Newton

A, Samama JP. 2002. Crystallographic and biochemical studies of DivK reveal novel features of an essential response regulator in *Caulobacter crescentus*. *J. Biol. Chem.* 277:42003–10

21. Hecht GB, Lane T, Ohta N, Sommer JM, Newton A. 1995. An essential single domain response regulator required for normal cell division and differentiation in *Caulobacter crescentus*. *EMBO J.* 14:3915–24
22. Hecht GB, Newton A. 1995. Identification of a novel response regulator required for the swarmer-to-stalked-cell transition in *Caulobacter crescentus*. *J. Bacteriol.* 177:6223–29
23. Hinz AJ, Larson DE, Smith CS, Brun YV. 2003. The *Caulobacter crescentus* polar organelle development protein PodJ is differentially localized and is required for polar targeting of the PleC development regulator. *Mol. Microbiol.* 47:929–41
24. Hoch JA, Varughese KI. 2001. Keeping signals straight in phosphorelay signal transduction. *J. Bacteriol.* 183:4941–49
25. Huguenel ED, Newton A. 1982. Localization of surface structures during procaryotic differentiation: role of cell division in *Caulobacter crescentus*. *Differentiation* 21:71–78
26. Hung DY, Shapiro L. 2002. A signal transduction protein cues proteolytic events critical to *Caulobacter* cell cycle progression. *Proc. Natl. Acad. Sci. USA* 99:13160–65
27. Ireland MM, Karty JA, Quardokus EM, Reilly JP, Brun YV. 2002. Proteomic analysis of the *Caulobacter crescentus* stalk indicates competence for nutrient uptake. *Mol. Microbiol.* 45:1029–41
28. Jacobs C, Ausmees N, Cordwell SJ, Shapiro L, Laub MT. 2003. Functions of the CckA histidine kinase in *Caulobacter* cell cycle control. *Mol. Microbiol.* 47:1279–90
29. Jacobs C, Domian IJ, Maddock JR, Shapiro L. 1999. Cell cycle-dependent polar localization of an essential bacterial histidine kinase that controls DNA replication and cell division. *Cell* 97:111–20
30. Jacobs C, Hung D, Shapiro L. 2001. Dynamic localization of a cytoplasmic signal transduction response regulator controls morphogenesis during the *Caulobacter* cell cycle. *Proc. Natl. Acad. Sci. USA* 98:4095–100
31. Jenal U, Fuchs T. 1998. An essential protease involved in bacterial cell-cycle control. *EMBO J.* 17:5658–69
32. Jensen RB, Wang SC, Shapiro L. 2002. Dynamic localization of proteins and DNA during a bacterial cell cycle. *Nat. Rev. Mol. Cell Biol.* 3:167–76
33. Jones SE, Ferguson NL, Alley MR. 2001. New members of the *ctrA* regulon: The major chemotaxis operon in *Caulobacter* is CtrA dependent. *Microbiology* 147:949–58
34. Kelly AJ, Sackett MJ, Din N, Quardokus E, Brun YV. 1998. Cell cycle-dependent transcriptional and proteolytic regulation of FtsZ in *Caulobacter*. *Genes Dev.* 12:880–93
35. Laub MT, Chen SL, Shapiro L, McAdams HH. 2002. Genes directly controlled by CtrA, a master regulator of the *Caulobacter* cell cycle. *Proc. Natl. Acad. Sci. USA* 99:4632–37
36. Laub MT, McAdams HH, Feldblyum T, Fraser CM, Shapiro L. 2000. Global analysis of the genetic network controlling a bacterial cell cycle. *Science* 290:2144–48
37. Leclerc G, Wang SP, Ely B. 1998. A new class of *Caulobacter crescentus* flagellar genes. *J. Bacteriol.* 180:5010–19
38. Lybarger SR, Maddock JR. 2001. Polarity in action: asymmetric protein localization in bacteria. *J. Bacteriol.* 183:3261–67
39. Marczynski GT, Lentine K, Shapiro L. 1995. A developmentally regulated chromosomal origin of replication uses essential transcription elements. *Genes Dev.* 9:1543–57
40. Marczynski GT, Shapiro L. 1992. Cell-cycle control of a cloned chromosomal origin of replication from *Caulobacter crescentus*. *J. Mol. Biol.* 226:959–77
41. Marczynski GT, Shapiro L. 2002. Control of chromosome replication in *Caulobacter*

crescentus. *Annu. Rev. Microbiol.* 56:625–56

42. Mohr CD, MacKichan JK, Shapiro L. 1998. A membrane-associated protein, FliX, is required for an early step in *Caulobacter* flagellar assembly. *J. Bacteriol.* 180:2175–85
43. Nierman WC, Feldblyum TV, Laub MT, Paulsen IT, Nelson KE, et al. 2001. Complete genome sequence of *Caulobacter crescentus*. *Proc. Natl. Acad. Sci. USA* 98:4136–41
44. Ohta N, Lane T, Ninfa EG, Sommer JM, Newton A. 1992. A histidine protein kinase homologue required for regulation of bacterial cell division and differentiation. *Proc. Natl. Acad. Sci. USA* 89:10297–301
45. Oke V, Long SR. 1999. Bacteroid formation in the *Rhizobium*-legume symbiosis. *Curr. Opin. Microbiol.* 2:641–46
46. Ouimet MC, Marczynski GT. 2000. Analysis of a cell-cycle promoter bound by a response regulator. *J. Mol. Biol.* 302:761–75
47. Poindexter JS. 1981. The caulobacters: ubiquitous unusual bacteria. *Microbiol. Rev.* 45:123–79
48. Quardokus E, Din N, Brun YV. 1996. Cell cycle regulation and cell type-specific localization of the FtsZ division initiation protein in *Caulobacter*. *Proc. Natl. Acad. Sci. USA* 93:6314–19
49. Quardokus EM, Din N, Brun YV. 2001. Cell cycle and positional constraints on FtsZ localization and the initiation of cell division in *Caulobacter crescentus*. *Mol. Microbiol.* 39:949–59
50. Quon KC, Marczynski GT, Shapiro L. 1996. Cell cycle control by an essential bacterial two-component signal transduction protein. *Cell* 84:83–93
51. Quon KC, Yang B, Domian IJ, Shapiro L, Marczynski GT. 1998. Negative control of bacterial DNA replication by a cell cycle regulatory protein that binds at the chromosome origin. *Proc. Natl. Acad. Sci. USA* 95:120–25
52. Reisenauer A, Quon K, Shapiro L. 1999. The CtrA response regulator mediates temporal control of gene expression during the *Caulobacter* cell cycle. *J. Bacteriol.* 181:2430–39
53. Reisenauer A, Shapiro L. 2002. DNA methylation affects the cell cycle transcription of the CtrA global regulator in *Caulobacter*. *EMBO J.* 21:4969–77
54. Robbins JR, Monack D, McCallum SJ, Vegas A, Pham E, et al. 2001. The making of a gradient: IcsA (VirG) polarity in *Shigella flexneri*. *Mol. Microbiol.* 41:861–72
55. Robertson GT, Reisenauer A, Wright R, Jensen RB, Jensen A, et al. 2000. The *Brucella abortus* CcrM DNA methyltransferase is essential for viability, and its overexpression attenuates intracellular replication in murine macrophages. *J. Bacteriol.* 182:3482–89
56. Rudner DZ, Pan Q, Losick RM. 2002. Evidence that subcellular localization of a bacterial membrane protein is achieved by diffusion and capture. *Proc. Natl. Acad. Sci. USA* 99:8701–6
57. Ryan KR, Judd EM, Shapiro L. 2002. The CtrA response regulator essential for *Caulobacter crescentus* cell-cycle progression requires a bipartite degradation signal for temporally controlled proteolysis. *J. Mol. Biol.* 324:443–55
58. Schmidt JM, Stanier RY. 1966. The development of cellular stalks in bacteria. *J. Cell Biol.* 28:423–36
59. Sciochetti SA, Lane T, Ohta N, Newton A. 2002. Protein sequences and cellular factors required for polar localization of a histidine kinase in *Caulobacter crescentus*. *J. Bacteriol.* 184:6037–49
60. Shapiro L, McAdams HH, Losick R. 2002. Generating and exploiting polarity in bacteria. *Science* 298:1942–46
61. Sheffery M, Newton A. 1981. Regulation of periodic protein synthesis in the cell cycle: control of initiation and termination of flagellar gene expression. *Cell* 24:49–57
62. Siam R, Marczynski GT. 2000. Cell cycle regulator phosphorylation stimulates two distinct modes of binding at a chromosome replication origin. *EMBO J.* 19:1138–47

63. Skerker JM, Shapiro L. 2000. Identification and cell cycle control of a novel pilus system in *Caulobacter crescentus*. *EMBO J.* 19:3223–34
64. Sommer JM, Newton A. 1989. Turning off flagellum rotation requires the pleiotropic gene *pleD: pleA*, *pleC*, and *pleD* define two morphogenic pathways in *Caulobacter crescentus*. *J. Bacteriol.* 171:392–401
65. Sommer JM, Newton A. 1991. Pseudoreversion analysis indicates a direct role of cell division genes in polar morphogenesis and differentiation in *Caulobacter crescentus*. *Genetics* 129:623–30
66. Steinhauer J, Agha R, Pham T, Varga AW, Goldberg MB. 1999. The unipolar *Shigella* surface protein IcsA is targeted directly to the bacterial old pole: IcsP cleavage of IcsA occurs over the entire bacterial surface. *Mol. Microbiol.* 32:367–77
67. Stephens C, Reisenauer A, Wright R, Shapiro L. 1996. A cell cycle-regulated bacterial DNA methyltransferase is essential for viability. *Proc. Natl. Acad. Sci. USA* 93:1210–14
68. Stephens CM, Shapiro L. 1993. An unusual promoter controls cell-cycle regulation and dependence on DNA replication of the *Caulobacter fliLM* early flagellar operon. *Mol. Microbiol.* 9:1169–79
69. Stephens CM, Zweiger G, Shapiro L. 1995. Coordinate cell cycle control of a *Caulobacter* DNA methyltransferase and the flagellar genetic hierarchy. *J. Bacteriol.* 177:1662–69
70. Stock AM, Robinson VL, Goudreau PN. 2000. Two-component signal transduction. *Annu. Rev. Biochem.* 69:183–215
71. Viollier PH, Sternheim N, Shapiro L. 2002. A dynamically localized histidine kinase controls the asymmetric distribution of polar pili proteins. *EMBO J.* 21:4420–28
72. Viollier PH, Sternheim N, Shapiro L. 2002. Identification of a localization factor for the polar positioning of bacterial structural and regulatory proteins. *Proc. Natl. Acad. Sci. USA* 99:13831–36
73. Wang SP, Sharma PL, Schoenlein PV, Ely B. 1993. A histidine protein kinase is involved in polar organelle development in *Caulobacter crescentus*. *Proc. Natl. Acad. Sci. USA* 90:630–34
74. Wheeler RT, Shapiro L. 1999. Differential localization of two histidine kinases controlling bacterial cell differentiation. *Mol. Cell* 4:683–94
75. Wolanin PM, Thomason PA, Stock JB. 2002. Histidine protein kinases: key signal transducers outside the animal kingdom. *Genome Biol.* 3:3013.1–8
76. Wu J, Ohta N, Newton A. 1998. An essential, multicomponent signal transduction pathway required for cell cycle regulation in *Caulobacter*. *Proc. Natl. Acad. Sci. USA* 95:1443–48
77. Wu J, Ohta N, Zhao JL, Newton A. 1999. A novel bacterial tyrosine kinase essential for cell division and differentiation. *Proc. Natl. Acad. Sci. USA* 96:13068–73
78. Zhuang WY, Shapiro L. 1995. *Caulobacter* FliQ and FliR membrane proteins, required for flagellar biogenesis and cell division, belong to a family of virulence factor export proteins. *J. Bacteriol.* 177:343–56
79. Zweiger G, Marczynski G, Shapiro L. 1994. A *Caulobacter* DNA methyltransferase that functions only in the predivisional cell. *J. Mol. Biol.* 235:472–85

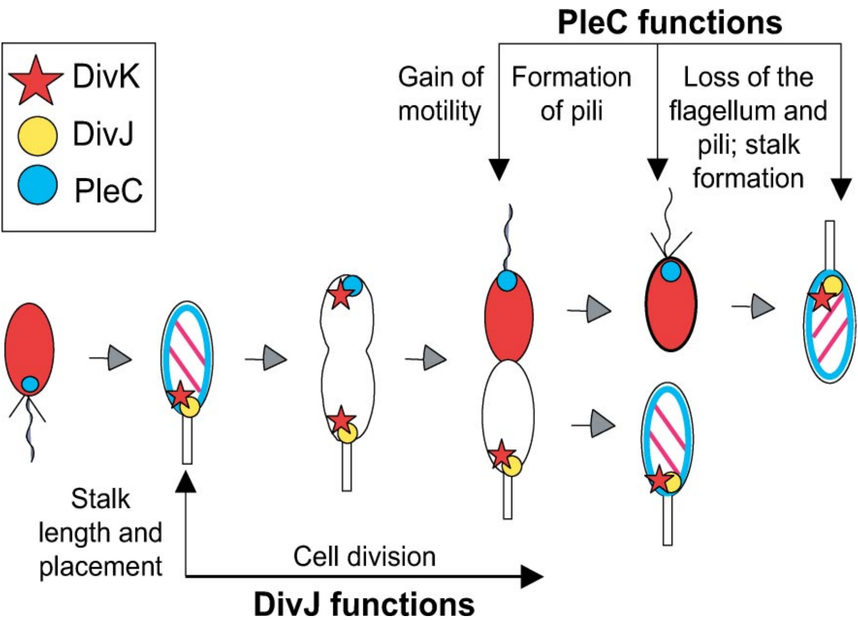

Figure 4 Cell cycle–dependent localization of the signal transduction proteins DivK, DivJ, and PleC.