Systems Biology of Caulobacter

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Abstract

The dynamic range of a bacterial species’ natural environment is reflected in the complexity of its systems that control cell cycle progression and its range of adaptive responses. We discuss the genetic network and integrated three-dimensional sensor/response systems that regulate the cell cycle and asymmetric cell division in the bacterium Caulobacter crescentus. The cell cycle control circuitry is tied closely to chromosome replication and morphogenesis by multiple feedback pathways from the modular functions that implement the cell cycle. The sophistication of the genetic regulatory circuits and the elegant integration of temporally controlled transcription and protein synthesis with spatially dynamic phosphosignaling and proteolysis pathways, and epigenetic regulatory mechanisms, form a remarkably robust living system.

Key Words

cell cycle, Caulobacter, regulation, genetic circuit
INTRODUCTION

In this review we focus on the top-level regulatory circuitry of the cell cycle control system in *Caulobacter crescentus* (“Caulobacter” hereafter). We discuss how genetic components that control the Caulobacter cell cycle regulatory system interact with each other and with the panoply of subsystems that implement the cell cycle.

Caulobacter is an aquatic α-proteobacterium that divides asymmetrically to produce differentiated progeny, a swarmer and a stalked daughter cell, each with distinct morphological features and regulatory programs. The daughter cells follow different developmental programs (Figure 1). The swarmer daughter cell has a single polar flagellum, polar chemotaxis receptors, and polar pili, and it does not initiate DNA replication until after a period of motility is completed (lasting less than 20% of the swarmer daughter cell’s generation time) and swarmer-to-stalked cell differentiation is initiated. This differentiation involves the loss of the flagellum and the polar chemotaxis receptors, retraction of the pili, construction of a stalk at the cell pole previously occupied by the flagellum, and initiation of chromosome replication. In contrast, the stalked daughter cell can initiate chromosome replication soon after cytoplasmic compartmentalization. As chromosome replication proceeds, the cell grows and becomes a predivisional cell while a pili secretion mechanism, a new flagellum, and the chemotaxis control subsystem are synthesized and assembled at the pole opposite the stalk. As chromosome replication is completing, the tubulin-like FtsZ-ring (the Z-ring) begins to constrict, and, after chromosome decatenation, the cytoplasm divides into two separate compartments inside the outer membrane about 20 min before completion of cell division (25, 26) (Figure 1b). Cytoplasmic compartmentalization initiates and enables divergent genetic programs in each chamber so that the next cell cycle effectively begins in each compartment at that moment (25, 30, 32).

FEATURES OF THE Caulobacter CELL

In the wild, *C. crescentus* is found in freshwater lakes and streams, where extended periods with low nutrient conditions are common and nutrients are highest near surfaces and floating bits of organic detritus. In this environment, decay products of plant degradation are common, and *Caulobacter* cells are equipped with environmental sensors, transporters, and metabolic pathways suitable to exploit metabolites originating from the breakdown of plant material (4, 17; M. Thanbichler, A. Iniesta & L. Shapiro, manuscript in preparation). The adaptation of Caulobacter to survival...
in oligotrophic conditions includes the ability to grow very slowly under low nutrient conditions and to halt cell cycle progression under conditions of carbon or nitrogen starvation (13; J. Lesley, et al., unpublished). A noncoding RNA mediates adaptation to carbon starvation. The end of the stalk has an adhesive holdfast that can attach the cell to surfaces, where a motile swarmer daughter cell is produced at each cell division (**Figure 1a**). This capability presumably facilitates maximum utilization of locally available nutrients.

The flagellated swarmer cell is capable of chemotaxis and dispersion from the site of the “parent” stalked cell. The stalk also greatly enhances the efficiency of environmental phosphate uptake, and, under low phosphate conditions, it adaptively grows longer to increase phosphate collection efficiency (43).

An oxygen-sensor/response network dynamically optimizes *Caulobacter* respiratory capacity over a wide range of O₂ concentrations (8). The cell surface is completely covered with a two-dimensional crystalline array of the RsaA protein (the S-layer) that is thought to act as a physical barrier to parasites (1). *Caulobacter* is capable of biofilm formation (11).

*Escherichia coli* can have overlapping rounds of DNA replication proceeding simultaneously to facilitate rapid growth, but *Caulobacter* regulates the initiation of replication so that there is one and only one chromosome replication event per cell cycle (29). Presumably, this is necessary because regulation of polar organelle development and cytokinesis is tightly integrated with the progression of the single round of replication.

This partial list of polar organelles and sensor/response capabilities illustrates *Caulobacter’s* adaptation to the challenges of its environment. The asymmetry of the daughter cells requires a cell cycle control mechanism that directs each daughter cell to initiate different developmental programs. In the remainder of the review, we focus on the mechanisms that implement this cell cycle.

**TOP-LEVEL SYSTEM ARCHITECTURE OF Caulobacter CELL CYCLE CONTROL**

The hierarchical cell cycle control system in *Caulobacter* has a core engine, a cyclical genetic circuit involving four master regulatory proteins (CtrA, GcrA, DnaA, and CcrM) that together control expression of at least 200 genes (6, 16, 18, 28; J. Collier & L. Shapiro, submitted) (**Figure 2**). The four proteins are synthesized in succession to drive the series of processive modular functions that execute the cell cycle (e.g., build the flagellum, replicate, separate, and methylate the chromosome, divide the cell, etc.). The overall design of the cell cycle control system includes epigenetic regulatory mechanisms, sensors, and signal transduction systems that provide feedback signals to synchronize the advance of the core engine with progression of chromosome replication and cytokinesis. The stabilities of CtrA, GcrA, and DnaA are actively controlled over the cell cycle (**Figure 3**). The molecular mechanisms of the pathway controlling CtrA proteolysis have recently been characterized (3, 21, 31), but those controlling GcrA and DnaA are largely unknown.

One of the cell cycle master regulators, CtrA, is an essential DNA-binding response regulator member of the two-component signal transduction family. CtrA is present in swarmer cells (**Figure 1b**), where it binds to the *Caulobacter* origin of replication (Cori) to silence replication initiation (37) while repressing *gcrA* transcription (16). CtrA∼P regulates the transcription of about 50 operons (95 genes) (28). In the nascent stalked cell compartment prior to cell separation and also during the swarmer-to-stalked cell transition, CtrA∼P is cleared from the cell by tightly controlled proteolysis (21, 31) so that the inhibition of DNA replication by CtrA is relieved. At this point in the cell cycle, newly synthesized DnaA synchronizes the start of chromosome replication with the phase of the cell cycle engine by simultaneously initiating replisome formation and activating *gcrA*
transcription (6, 18) (Figure 2). This positioning of DnaA within the cell cycle circuit is exploited to halt the cell cycle under conditions of nutrient deprivation by accelerating DnaA proteolysis to remove it from the cell (13). [Proteolysis of the Caulobacter DnaA protein is dynamically regulated (13), whereas the Escherichia coli DnaA protein is very stable (41); this cell cycle regulation of the amount of DnaA in the cell may have evolved in response to the necessity to avoid reinitiation of DNA replication in Caulobacter.] GcrA activates the ctrA P1 promoter, the first of the two ctrA promoters to be consecutively activated during the cell cycle (Figure 2). Since GcrA is probably not a transcription factor, it likely affects ctrA P1 indirectly (16, 38). Then, as soon as CtrA>P1 begins to accumulate, the ctrA P1 promoter is activated by positive autoregulation, the amount of CtrA>P1 in the cell increases rapidly, and the ctrA P1 promoter is repressed (Figure 2). This positive feedback loop in ctrA regulation creates a binary switch element in the Caulobacter cell cycle. As long as this feedback loop is not interrupted, the amount of CtrA>P1 in the cell remains high. Another multistage feedback loop, culminating in the CckA→ChpT phospho-signal pathway (described below), must remain active for the ctrA P1 promoter feedback loop.
Stability of the four master regulators in the core cell cycle engine (green background) is dynamically controlled. The pathways controlling the proteases that determine the stability have only been identified for CtrA (21, 31), and, in the case of GcrA, the protease itself is not known. The CpdR single domain response regulator is required to localize the ClpXP protease to the cell pole where it degrades CtrA in the presence of the RcdA localization factor. The mechanisms connecting CckA to CtrA dephosphorylation and proteolysis have been characterized (3, 21), but the pathways leading to CckA localization are not known. There are intriguing observations suggesting another oscillatory pathway from CtrA∼P to CckA deactivation via the DivK single-domain response regulator (see Figure 2 and the text). Dynamic localization of proteins in the CckA→ChpT→(CtrA, CpdR) pathway is an essential element in its operation. Question marks indicate unknown factors.

When the CckA→ChpT pathway is inhibited, either in the nascent stalked cell compartment at the time of cytoplasmic compartmentalization or at the swarmer-to-stalked cell transition (3), CtrA∼P stops being phosphorylated, and it is degraded so that active CtrA∼P is rapidly cleared from the cell, allowing the initiation of DNA replication.

It has been proposed that the differentiation of the swarmer cell into a stalked cell occurs when the phosphosignal originating from the CckA kinase is eliminated (3), so that CtrA stops being phosphorylated and CtrA degradation occurs (21, 22). Chromosome replication can initiate after CtrA∼P is eliminated in the stalked cells created by differentiation of swarmer cells or in the nascent stalked cell compartment created when the cytoplasm separates into two compartments [about 20 min before the cell separates into swarmer and stalked progeny cells (25)].
As noted above, the protein concentrations of the master regulators, CtrA, GcrA, and DnaA (Figures 1b and 2), drive creation and operation of many modular subsystems that implement cell cycle progression. Epigenetic control via DNA methylation catalyzed by the CcrM DNA methyltransferase is central to the control of regulatory genes whose expression depends on their promoter methylation state (see below). Since crm expression is activated by CtrA–P (36, 38), the timing of CcrM synthesis, and thus of DNA remethylation, depends on timing of CtrA expression and its activation by phosphorylation. The ctrA P1 promoter, which initiates resynthesis of CtrA, is activated indirectly by GcrA, but only when it is in the hemimethylated state, after the replication fork passes the ctrA gene (39) (Figure 2). CcrM is synthesized during a short interval near the end of DNA replication, and then remethylates the genome within about 20 min before it is inactivated.

(DnaA GcrA CtrA CcrM

DNA Replication

Cyto-kinesis

Initiation Elongation Blocks reinitiation Remethylates chromosome

Compartmentalization starts CtrA proteolysis & dephosphorylation in stalked cell compartment

Figure 4

Multiple pathways synchronize progression of the core cell cycle engine (green background) with implementation of cell cycle modular functions. DnaA’s dual role in initiating DNA replication and GcrA synthesis synchronizes cell division with DNA replication at the start of S-phase. GcrA activates replisome proteins. CtrA–P blocks reinitiation of DNA replication and activates proteins required for cytokinesis and polar development in the predivisional cell. At the end of chromosome replication, CcrM remethylates methylation-regulated promoter sites (Figure 2) to reset the promoters for the next cycle. CtrA phosphorylation arrest and CtrA degradation in the stalked cell compartment (central to the asymmetric cell cycle) are triggered by cytoplasmic compartmentalization about 20 min before cell separation.

(2). Chromosome replication and cytokinesis are processive reactions whose progress determines the rate of cell cycle progression. Signals indicating progress of these and other reactions feed back into the cell cycle engine and pace its forward motion (Figure 4).

A critical element of the Caulobacter cell cycle regulatory circuit is a link between dnaA expression and the progression of chromosome replication that helps synchronize the cell cycle engine with cell cycle events. The dnaA gene is located next to the origin of replication, Cori, and the dnaA promoter region contains two DNA methylation sites. The synthesis of DnaA is coordinated with its function by an epigenetic mechanism, which depends on the methylation state of the dnaA promoter (J. Collier & L. Shapiro, submitted). The chromosomal location of dnaA, near the Cori, also contributes to control of the DnaA concentration over the cell cycle, since the transcription of dnaA is efficient when the dnaA promoter is in the fully methylated state prior to the initiation of replication, but inefficient when the dnaA promoter becomes hemimethylated upon passage of the replication fork, right after the initiation of DNA replication.

SPATIAL ORGANIZATION OF CELL CYCLE ENGINE COMPONENTS

Dynamic subcellular localization of regulatory proteins during the cell cycle is an essential part of Caulobacter cell cycle control. Localized components of the core cell cycle circuit that are at specific positions within the cell at specific stages of the cell cycle include members of the two-component signal transduction family, the ClpXP protease, and factors that mediate localization of regulatory and structural proteins. Protein localization serves two functions: One is to ensure that polar organelles assemble at the correct pole at the correct cell cycle stage. For example, the single flagellum and the pili secretion apparatus are assembled at the pole of the...
predivisional cell opposite the pole bearing the stalk. When the swarmer cell differentiates into a stalked cell, a new stalk is assembled at the site previously occupied by the flagellum (Figure 1b). Another function of regulatory protein localization is construction of a spatially distributed multiprotein switching mechanism that causes differential phosphorylation of CtrA in the two cytoplasmic compartments established late in the cell cycle by inner-membrane fission. Thereafter, the strong dependence of CtrA DNA-binding affinity on its phosphorylation state leads to implementation of different developmental programs in each nascent daughter cell.

**Polar Organelle Positioning**

The predivisional cell bears a flagellum and several pili at one cell pole and a stalk at the opposite pole (Figure 1b). The two new cell poles formed at cell division each bear a marker protein, TipN, that marks the site for new flagellum biogenesis (19, 27). A cyclic di-GMP phosphodiesterase homolog, TipF, which is required for flagellar assembly, is colocalized with TipN (19). If TipN is provided ectopically, cells branch and form new poles, suggesting that the polar-localized TipN contributes to establishing the polar axis of the cell (27). It has also been reported that TipN determines the polar positioning of the PleC histidine kinase at the flagellar cell pole (27). Correct positioning of PleC is required for polar pili biogenesis (42). Tracking of single PleC-GFP molecules in vivo suggests that PleC-GFP is positioned at the correct pole by a diffusion/capture mechanism (9).

PodJ is a second polar localization factor (15, 42). The full-length PodJ protein, PodJL, is synthesized late in the stalked cell stage and positioned at the incipient swarmer pole where it mediates positioning of PleC and the CpaE component of the pili-specific secretion apparatus to the same pole. After cytokinesis, PodJL is clipped to the shorter PodJS form that resides at the flagellar pole, where it is required for chemotaxis and holdfast formation (15, 42).

**A Spatially Distributed Switch Controls Asymmetric Development**

The temporally controlled synthesis, phosphorylation, and proteolysis of CtrA are orchestrated by redundant regulatory pathways that involve dynamic cellular localization of both phosphosignaling proteins and a protease complex.

The hybrid histidine kinase CckA is localized to the cell poles and strongly autophosphorylated following the initiation of DNA replication (3, 23). The signal(s) that activate CckA localization and its autophosphorylation at a specific stage in the cell cycle are not yet known. The phosphoryl group on the localized, autophosphorylated CckA is transferred intramolecularly to its C-terminal receiver domain. The cytoplasmic histidine phosphotransferase ChpT is then phosphorylated by the CckA receiver domain (3). ChpT in turn phosphorylates (i) newly synthesized CtrA (3) and (ii) the single-domain response regulator CpdR (3). Unphosphorylated CpdR promotes CtrA degradation by mediating the polar localization of the ClpXP protease to the stalked pole of the new stalked cell (21). In the absence of CpdR, the ClpXP protease is not localized to the cell pole and CtrA is not degraded. The RcdA localization factor that binds to both CtrA and ClpX is required for CtrA interaction with the polar ClpXP protease, contributing to the critical temporal specificity of CtrA proteolysis (31). Note, however, that in vitro ClpXP rapidly degrades CtrA with or without RcdA (5), suggesting that RcdA is not a typical adaptor for CtrA proteolysis, but that its function is to bring CtrA and ClpXP into close proximity at the cell pole. An important challenge is to determine the molecular mechanism for polar localization of the ClpXP protease and how localization influences its activity. The CckA/ChpT-mediated phosphorylation of CpdR leads to release of both CpdR-P and
the ClpXP protease from the cell pole, and cessation of CtrA degradation (21). Thus, the autophosphorylation of polar CckA leads simultaneously to the phosphorylation of CtrA and to its protection from proteolysis by the phosphorylation of CpdR, ensuring robust CtrA∼P activity. Conversely, the down-regulation of CckA activity by loss of its polar position results in the degradation of CtrA prior to S-phase (3), an event that is critical to cell cycle progression.

So long as ChpT is in diffusive contact with the active and localized CckA kinase, CtrA phosphorylation is maintained. Breaking the CckA→ChpT→(CtrA, CpdR) phospho-cascade leads to (i) accumulation of unphosphorylated CtrA and (ii) localization of ClpXP followed by CtrA degradation. These phospho-cascades are physically interrupted in the nascent stalked cell compartment when inner membrane fission compartmentalizes the cell during cytokinesis. Thereafter, CtrA∼P is maintained in the swarmer cell compartment, but rapidly degraded in the stalked cell compartment (10, 26). The resulting spatial asymmetry in CtrA∼P causes differential expression of 95 genes in the nascent swarmer and stalked cell compartments.

Down-regulation of the CckA→ChpT→(CtrA, CpdR) phospho-cascade is also a principal event in the swarmer-to-stalked cell transition. The essential single-domain response regulator DivK has been implicated in regulating the loss of CckA autophosphorylation (3). In a divK cold-sensitive mutant, CckA activity is elevated, resulting in elevated phosphorylation of both CtrA and CpdR (3), so that high concentrations of CtrA∼P are maintained (3), resulting in a G1-arrest (20). Consistent with this observation, a loss-of-function mutation in divJ, which encodes the cognate kinase for DivK, is suppressed by mutations that lower CckA kinase activity (35).

The mechanism whereby DivK disrupts CckA autophosphorylation and localization is not known, but it likely involves control of the phosphorylation state of DivK (3). The phosphorylation state of DivK is controlled by the DivJ histidine kinase, by the PleC phosphatase (14, 44, 45), and possibly other sensor kinases. In predivisional cells, DivJ localizes to the stalked pole, and PleC localizes to the swarmer pole (44). During cytokinesis, cytoplasmic compartmentalization thus leads to differential inheritance of these proteins: The nascent stalked cell compartment inherits DivJ and would accumulate DivK∼P (30), whereas the nascent swarmer cell compartment would inherit PleC and decrease phosphorylated DivK. In a DivK cold-sensitive mutant, CckA and CtrA phosphorylation is high, so the differential localization of DivK’s kinase and phosphatase in the predivisional cell would thereby contribute to the asymmetry of CtrA activity and stability in the two daughter cells. The swarmer cell progeny would retain high CtrA activity, whereas in the stalked cell progeny, DivK∼P would contribute to the loss of CtrA∼P, thus enabling initiation of DNA replication. In this model, the physical compartmentalization of the cytoplasm in late predivisional cells and the asymmetric inheritance of DivJ and PleC would thus be translated into the differential regulation of CtrA in the two daughter cells.

CtrA∼P activates transcription of divK in late predivisional cells and new synthesis of DivK occurs mainly in predivisional cells (20, 28). Thus, CtrA∼P appears to stimulate its own destruction by inducing the synthesis of DivK, which then feeds back to down-regulate CtrA activity via the CckA→ChpT→(CtrA, CpdR) phospho-cascade. This, and possibly other, negative feedback loop plays an important role in the cell cycle control system. In this model, two separate cyclical subcircuits act together to regulate cell cycle progression in Caulobacter. One, involving the master regulators GcrA, DnaA, and CcrM, produces cyclical expression of ctrA transcription (Figure 2). The other, involving DivK, CckA, ChpT, and CpdR, produces cyclical CtrA activity. It has been proposed that the cell cycle of the yeast *Saccharomyces cerevisiae* is driven by
two separable, but interlaced, oscillators (7). Additional feedbacks must also control the cell cycle-dependent stability of DnaA and GcrA (Figure 3).

**SIGNALING SYSTEMS**

A systematic analysis of deletion strains for each of the 106 two-component signaling genes (62 histidine kinases and 44 response regulators) identified 39 deletions with a cell cycle, growth, or morphogenesis phenotype (40). However, these deletion strains were analyzed in rich media. Many of the genes that are dispensable for mid-log phase growth in rich media may be crucial for responding to various environmental signals or alternative growth conditions. Eight two-component signaling genes, for example, are necessary for growth in minimal media, but dispensable during growth in rich media (M.T. Laub, unpublished data). The two-component system FixL-FixJ is required for growth specifically in hypoxic conditions (8). In response to oxygen limitation, the FixL-FixJ system up-regulates genes encoding high-affinity terminal oxidases to cope with less dissolved oxygen in the environment. An orthologous signaling system in *S. meliloti* up-regulates genes required for nitrogen fixation (12). Thus, different bacterial species use the same two-component signaling pathways differently to produce responses tailored to the demands of their specific ecological niche.

In addition to the 106 genes encoding members of the two-component signal transduction family, many of which regulate cell cycle progression and asymmetry, the *Caulobacter* genome also encodes several other large, paralogous families of signaling and regulatory proteins (33). For instance, the *Caulobacter* genome is predicted to encode more than 150 transcription factors, in addition to the response regulators that have DNA-binding domains (33). Many of these transcription factors presumably control environmental responses, although others may be required for cell cycle regulation.

Signal transduction using the small molecule cyclic-di-GMP has recently been recognized as a major signaling modality throughout the bacterial kingdom (24). Diguanylate cyclases and phosphodiesterases catalyze the synthesis and destruction, respectively, of cyclic-di-GMP. Although it is not known how cyclic-di-GMP effects changes in cellular behavior, the prevalence of these signaling systems suggests that they are components of bacterial regulatory circuits. *Caulobacter* encodes four predicted diguanylate cyclases, three predicted phosphodiesterases, and seven proteins with both domains. One of the *Caulobacter* diguanylate cyclases, PleD, regulates polar development during cell cycle (34). Both the enzymatic activity and subcellular localization of PleD are controlled by the phosphorylation of its response regulator-like receiver domain. Diguanylate cyclases often have this configuration, acting as the output domain of a response regulator, underscoring the highly interconnected nature of signaling pathways in bacteria. Mapping the connectivity, as well as the inputs and outputs, of its signaling pathways will be crucial to a systems-level understanding of any bacterial species.

**THE BACTERIAL CELL IS AN INTEGRATED SYSTEM**

A major lesson from the recent discoveries about the *Caulobacter* cell cycle control system is that the bacterial cell has to be studied and described as a three-dimensional integrated system. We are not surprised that the core control circuitry is closely synchronized with cell development, but the epigenetic role of promoter methylation state that ties synchronization to the replication of the chromosome and the strong coupling with the cell’s 3D topology are new revelations. There are many important feedback pathways in the core engine, particularly those controlling master regulator stability (Figure 3) that we do not yet understand.
We also have to remember that the cell cycle processes underlying most of our investigations are those of a cell growing in log phase. However, the cell cycle control system has to deal with a much larger range of situations. There are specialized sensor response systems or cell design features for dealing with varying supplies of every nutrient the cell requires, for responding to the environmental situation (osmolarity, pH, temperature, nutrient availability), and for responding to (or evading) predators and competitors. In each case, but especially for varying nutrient environments, the progression of the cell system has to adapt quickly to the situation. Part of *Caulobacter*’s strategy for surviving low and variable nutrient environments appears to involve the ability to operate successfully over a wide range of cell cycle progression rates. We are just beginning to understand the mechanisms whereby the cell responds to the full range of nutrient limitations. Both nitrogen and carbon starvation lead to DnaA degradation, and thus to cessation of cell cycle progress (13). Low oxygen elicits not only activation of alternate pathways for oxygen metabolism, but other less well understood cell-wide responses (8). Many challenges remain, including identification of the pathways that couple progression of the cell cycle engine with stability and activity of the master regulators DnaA, GcrA, and CtrA. Recently, about 30 small RNAs have been identified, of which one, the CrfA noncoding RNA, is a critical player in the cell’s adaptation to glucose starvation (S. Landt, J. Lesley, E. Abeliuk, P.T. McGrath, L. Shapiro & H.H. McAdams, unpublished data; J. Lesley, et al., unpublished). The regulatory roles of sRNAs and their contribution to adaptive responses of the cell cycle control circuit to nutrient limitations are just now being explored.

### SUMMARY POINTS

1. The core of the cell cycle control system in *Caulobacter crescentus* is a cyclical genetic circuit involving four master regulatory proteins (CtrA, GcrA, DnaA, and CcrM) that are synthesized in succession to control expression of at least 200 genes.

2. The overall cell cycle control system design includes epigenetic regulatory mechanisms, sensors, and signal transduction systems that provide feedback signals to synchronize the advance of the core engine with progression of chromosome replication and cytokinesis.

3. Dynamic subcellular localization of regulatory proteins during the cell cycle is an essential part of *Caulobacter* cell cycle control.

4. Multiple feedback loops produce cell cycle-dependent proteolysis of the CtrA, GcrA, and DnaA master regulators.

5. CtrA appears to stimulate its own destruction by inducing the synthesis of DivK, which then down-regulates CtrA activity via a phospho-cascade, so that at least one additional cyclical subcircuit acts with the core oscillator to regulate cell cycle progression in *Caulobacter*.

6. Regulation of the bacterial cell has to be studied and described as a three-dimensional integrated system.
FUTURE ISSUES

1. Determine the pathways and molecular mechanisms for polar localization of proteins.
2. Determine all pathways and molecular mechanisms for dynamic control of regulatory protein stability.
3. Determine the signals that sensor kinases use to monitor cell cycle progression.
4. Determine the sensors and signaling pathways that connect environmental circumstances to cell cycle progression.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

LITERATURE CITED


**Describes a novel method for identifying cognate histidine kinases and response regulators.**