

## Extra Views

# A Genetic Oscillator and the Regulation of Cell Cycle Progression in *Caulobacter crescentus*

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## KEY WORDS

cell cycle, bacteria, *Caulobacter*, genetic circuit, CtrA, GcrA

## ABSTRACT

Analyses of cell polarity, division, and differentiation in prokaryotes have identified several regulatory proteins that exhibit dramatic changes in expression and spatial localization over the course of a cell cycle. The dynamic behavior of these proteins is often intrinsically linked to their function as polarity determinants.<sup>1-3</sup> In the  $\alpha$ -proteobacterium, *Caulobacter crescentus*, the CtrA global transcriptional regulator exhibits a spatially and temporally dynamic expression pattern across the cell cycle. CtrA plays key roles in asymmetric cell division and in the timing of chromosome replication.<sup>3,4</sup> An additional global regulator, GcrA, has recently been discovered that both regulates and is regulated by CtrA.<sup>5</sup> Together, these regulatory proteins create a genetic circuit in which the cellular concentrations of CtrA and GcrA oscillate spatially and temporally to control daughter cell differentiation and cell cycle progression.

## CELL CYCLE PROGRESSION AND DIMORPHISM IN *CAULOBACTER CRESCENTUS*

In *C. crescentus*, a tightly orchestrated signaling network controls asymmetric cell division yielding two developmentally-distinct daughter cell types: (1) swarmer cells that possess a single polar flagellum and pili and whose chromosome replication is inhibited, and (2) stalked cells that possess a thin polar stalk capped with an adhesive holdfast that actively replicate their chromosome (Fig. 1A). The dimorphic nature of *Caulobacter* permits isolation of a homogenous population of swarmer cells by means of density gradient centrifugation.<sup>6</sup> These synchronized swarmer cells can then be tracked through the cell cycle as they first shed their polar flagellum and pili and then grow a stalk at the same pole. The newly-formed stalked cells subsequently create chemotaxis control circuitry and build a flagellum and pili secretion complex at the pole opposite the stalk, which becomes a pole of the nascent daughter swarmer cell (Fig. 1A). Thus the complement of polar organelles at the *Caulobacter* cell poles is radically changed during the course of a cell cycle, with each pole fated to first grow a flagellum and then a stalk.

The timing of chromosome replication is also tightly controlled during the cell cycle. The phosphorylated form of the CtrA response regulator (CtrA-P) binds to the chromosomal origin of replication in the swarmer cell, repressing initiation of replication (Fig. 1A). Shortly after the swarmer cell's flagellum and pili are shed, CtrA-P is proteolyzed<sup>7</sup> by the ClpXP protease<sup>8</sup> permitting initiation of chromosome replication during the stalked and predivisive phases of the cell cycle. A restrictive barrier forms between the incipient daughter cells following the completion of chromosome partitioning to the two halves of the predivisive cell, but well before cell separation.<sup>9</sup> As soon as two distinct compartments are formed, CtrA-P is proteolyzed in the daughter stalked cell<sup>7</sup> but not in the daughter swarmer cell where the continued presence of CtrA-P represses initiation of chromosome replication. The stalked cell immediately initiates and new round of DNA replication, while the swarmer cell must first differentiate into a stalked cell as described above to reinitiate the cell cycle. Global transcriptional analysis of CtrA mutants<sup>10</sup> has shown that CtrA not only acts as an origin inhibitor, but also regulates genes required for cell division, DNA methylation, polar flagellar and pili biogenesis, and chemotaxis.

## CTR A AND GCRA ARE OSCILLATING COREGULATORS OF THE *CAULOBACTER* CELL CYCLE

CtrA is removed from early stalked cells by proteolysis, then is resynthesized to high levels in late-predivisive and swarmer cells<sup>7</sup> (Figs. 1A and B). This changing level of

CtrA is key to its role as a silencer of the origin of DNA replication in swarmer cells and as an activator of the gene encoding the CcrM DNA methyltransferase and flagellar and chemotaxis genes in predivisional cells.<sup>10-12</sup> The striking temporal and spatial oscillatory pattern of CtrA concentration during the cell cycle raises the question of what regulatory factors control CtrA expression and activity. It is known that regulated proteolysis by ClpXP<sup>8</sup> plays an important role by rapidly decreasing CtrA concentration at the swarmer-to-stalked cell transition. Another layer of CtrA regulation is provided by the essential histidine kinase CckA, which directly phosphorylates CtrA to the CtrA-P form,<sup>13</sup> thereby serving as a post-translational regulator of its DNA-binding activity<sup>14</sup> (Fig. 2). Additional regulation occurs at the P1 and P2 promoters of *ctrA* itself, with CtrA serving as an auto-activator of its P2 promoter and a repressor of its P1 promoter<sup>15</sup> (Fig. 2). Transcription of *ctrA* in stalked cells begins from the P1 promoter shortly after DNA replication initiates. The positive feedback loop between CtrA and its P2 promoter upregulates *ctrA* transcription in predivisional cells<sup>15</sup> (Fig. 2). The methylation state of the chromosome introduces yet another layer of regulation. Specifically, the *ctrA* P1 promoter is repressed when the chromosome is fully methylated, and accessible for activation only when hemi-methylated.<sup>16</sup> Replication initiates on a fully methylated chromosome, and as the replication fork passes the *ctrA* gene two hemi-methylated copies are generated, thus priming *ctrA* for transcriptional activation. The essential methyltransferase CcrM,<sup>17</sup> which is under positive transcriptional control by CtrA, subsequently silences the P1 promoter as it remethylates the chromosome in the late predivisional cell.

While the mechanisms described above partially explain how the concentration of CtrA is controlled throughout the cell cycle, until recently no factor had been attributed to the upregulation of *ctrA* transcription in stalked and predivisional cells. Now studies of a temperature sensitive *Caulobacter* mutant by Holtzendorff and colleagues<sup>5</sup> have identified another essential gene, *gcrA*, which acts as a positive regulator of *ctrA* and that is itself repressed by CtrA. *gcrA* encodes a 190 amino acid protein that is conserved among the  $\alpha$ -proteobacteria but is not found in other families of sequenced prokaryotes or eukaryotes. GcrA is most highly expressed in stalked and early predivisional cells. GcrA positively regulates transcription of genes required for maintenance of cellular asymmetry such as the localization factor, *podJ*<sup>18</sup> and the histidine kinase *pleC*<sup>19</sup> as well as genes required for formation of the replication machinery. Importantly, GcrA also activates transcription of *ctrA* from its hemi-methylated P1 promoter, which is created by the passage of the replication fork (Fig. 2). This ties activation of *ctrA* transcription by GcrA to a specific time in the progression of DNA replication. A chromatin immunoprecipitation assay revealed an interaction between GcrA and the promoter regions of several genes including the replication initiation factor *dnaA*, the polarity regulator *podJ*, and *ctrA*. It is not known if these promoter interactions are direct or are mediated by another protein. Notably, alignment of GcrA against the Conserved Domain Database<sup>20</sup> reveals no homology to known DNA-binding domains. Thus, if the interaction with DNA

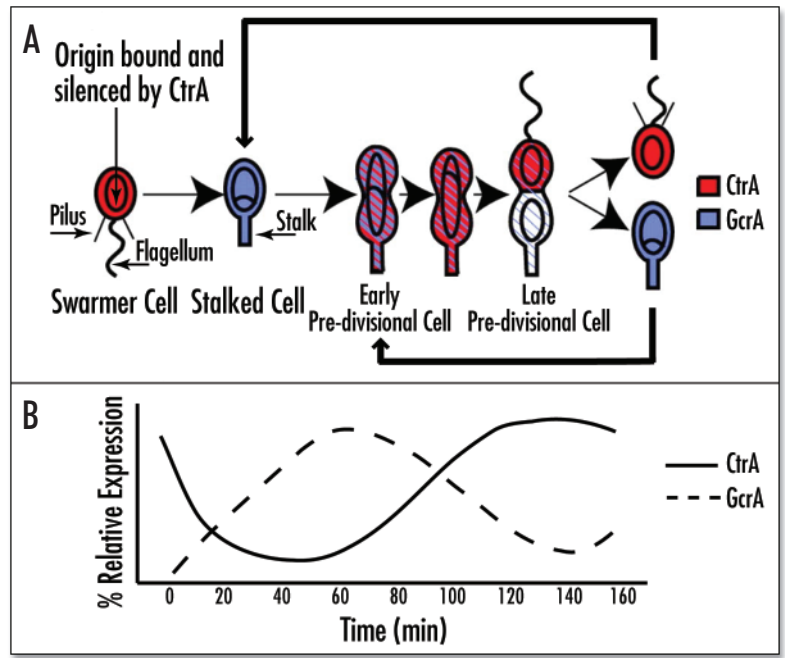


Figure 1. (A) Cartoon of the *Caulobacter crescentus* cell cycle. CtrA is shown in red and GcrA in blue. The chromosome is shown as a circle inside the cell with the theta-like structure representing replicating DNA. (B) Protein levels of CtrA (solid line) and GcrA (dashed line) over the course of the cell cycle.

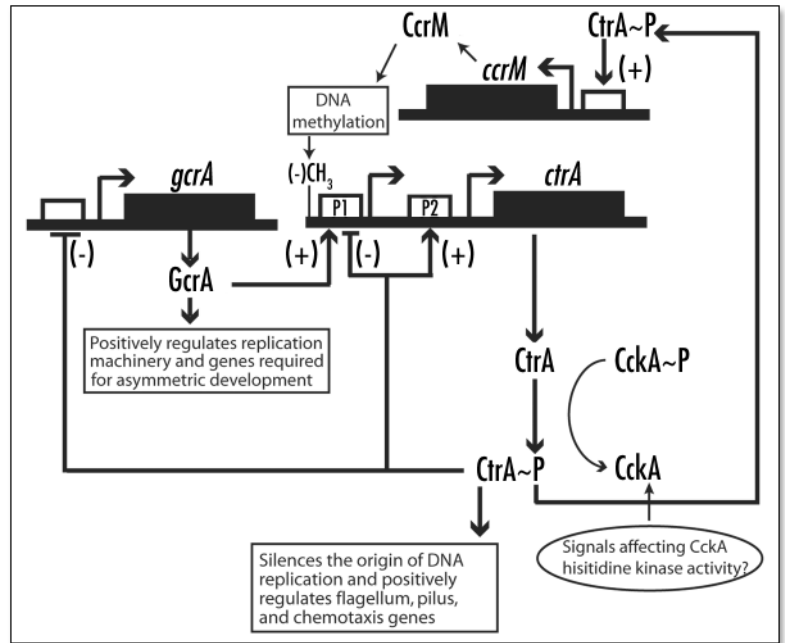


Figure 2. Model of the CtrA/GcrA genetic circuit.

is direct, GcrA almost certainly binds DNA in a novel way.

The oscillatory behavior of the CtrA/GcrA genetic circuit arises because GcrA acts as a positive regulator of CtrA, while CtrA acts as a negative regulator of GcrA. The CtrA/GcrA genetic circuit shown in Figure 2, results in spatial and temporal separation of expression of these global regulators over the course of a cell cycle.<sup>5</sup> These out-of-phase oscillations of the CtrA and GcrA master regulators (Fig. 1B) drive the progression of the *Caulobacter* cell cycle. In

particular, the presence of CtrA-P in the swarmer cell inactivates DNA replication by blocking the origin of replication and repressing GcrA expression. At the swarmer-to-stalked cell transition, CtrA-P is proteolyzed by ClpXP in response to an unknown signal. Upon degradation of CtrA, *gcrA* is derepressed allowing its gene product to activate transcription of genes required for DNA replication and polar morphogenesis as well as transcription of *ctrA* when the hemi-methylated promoter appears. The subsequent increase in CtrA concentration renews repression of *gcrA* in predivisive and swarmer cells.

## ADDITIONAL REGULATORY PATHWAYS COUPLED TO THE CTRA/GCR A CIRCUIT

The oscillatory CtrA/GcrA feedback circuit described above is a central element of the regulation of the *Caulobacter* cell cycle and asymmetric cell division. Yet, even though these two master regulatory proteins have been shown to directly control some 145 genes, it is clear there must be one or more additional top-level master regulators. One indicator of these missing regulatory proteins is the presence of numerous other cell cycle-regulated genes that do not appear to be regulated by either CtrA or GcrA (as one example, several components of the DNA replication complex). In addition, there are numerous status reporting signals from various cell cycle processes to the top-level CtrA/GcrA circuit. Examples include signals indicating progress of cell compartmentalization<sup>21</sup> and progress in construction of polar organelles. When cells encounter environmental stress conditions or sustain DNA damage, signals to halt or slow cell cycle progression are activated.

Sensory/signaling pathways that tie the intra- and extracellular environment into cell cycle regulation remain largely unexplored. In its natural dilute freshwater habitat, *Caulobacter* normally experiences a wide range of physical and chemical conditions to which it must adapt to survive. With sixty-one sensor histidine kinases annotated in its genome and a total of 105 two-component regulatory proteins,<sup>22</sup> *Caulobacter* appears to be well equipped to respond to its environment. However, function has only been assigned to a handful of these sensory proteins and the environmental signals affecting *Caulobacter* kinase activity remain uncharacterized. Environmental signals (e.g., oxygen binding, light detection, redox change, small molecule binding, etc.) may feed into the pathway controlling CckA kinase activity to affect the phosphorylation state and DNA-binding activity of CtrA. Analysis of wild-type and two-component mutant strains of *Caulobacter* grown under a variety of physiological conditions will provide insight into how cell cycle progression is modulated by changes in the nutrient availability, oxygen concentration, or the presence of other physical and chemical signals. Certainly, we have only begun to understand the regulatory systems underlying cell cycle progression in this “simple” bacterial cell.

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