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Sensory domain of the cell cycle kinase CckA regulates the differential DNA binding of the master regulator CtrA in *Caulobacter crescentus*

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A B S T R A C T

Sophisticated signaling mechanisms allow bacterial cells to cope with environmental and intracellular challenges. Activation of specific pathways ameliorates these challenges and thereby warrants integrity. Here, we demonstrate the pliability of the CckA-CtrA two-component signaling system in the freshwater bacterium *Caulobacter crescentus*. Our forward genetic screen to analyze suppressor mutations that can negate the chromosome segregation block induced by the topoisomerase IV inhibitor, NstA, yielded various point mutations in the cell cycle histidine kinase, CckA. Notably, we identified a point mutation in the PAS-B domain of CckA, which resulted in increased levels of phosphorylated CtrA (CtrA∼P), the master cell cycle regulator. Surprisingly, this increase in CtrA∼P levels did not translate into a genome-wide increase in the DNA occupancy of CtrA, but specifically enriched its affinity for the chromosomal origin of replication, C_{ori}, and for a very small subset of CtrA regulated promoters. We show that through this enhanced binding of CtrA to the C_{ori}, cells are able to overcome the toxic defects rendered by stable NstA through a possible slow down in the chromosome replication cycle. Taken together, our work opens up an unexplored and intriguing aspect of the CckA-CtrA signal transduction pathway. The distinctive DNA binding nature of CtrA and its regulation by CckA might also be crucial for pathogenesis because of the highly conserved nature of the CckA-CtrA pathway in alphaproteobacteria.

1. Introduction

Bacteria harbor robust signaling mechanisms to tolerate stressful conditions. Exquisitely fine-tuned regulatory cascades in bacteria impart their effect to regulate development in response to changes in the internal or external milieu. The aquatic α-proteobacterium, *Caulobacter crescentus* (henceforth *Caulobacter*), has emerged as a powerful model organism for studying the complex signaling mechanisms that control cell cycle and development in response to environmental cues. During its cell cycle, *Caulobacter* undergoes asymmetric division to produce progenies with distinct developmental fates. One of the daughter cells, the swarmer cell, is motile and its locomotion is assisted by the polar flagellum [1,2]. In contrast, the stalked daughter cell is sessile and capable of replicating its chromosome [3,4]. The G1-like swarmer cell has to terminally differentiate into a stalked cell to enter into the proliferative phase. This G1 to S-like transition is marked by the shedding of the flagellum, retraction of the pili, and production of a stalk at the same cell pole.

In the swarmer cells, the master transcriptional regulator, CtrA, inhibits the DNA replication. The *Caulobacter* origin of replication, C_{ori}, is bound by CtrA, which prevents replisome formation in the swarmer cells [5]. Concurrent with the swarmer to stalked cell transition, CtrA is degraded by the ClpXP protease [6] thus allowing the binding of DnaA, the replication initiator, to the C_{ori} triggering chromosome replication [7]. Apart from blocking DNA replication initiation, CtrA also serves as a transcription factor to drive the expression of numerous developmentally important genes in a cell cycle-dependent manner [8].

The differential activity of CtrA in the swarmer and stalked cells is of paramount significance for generating different cell fates. Multiple levels of regulation involving control at the level of synthesis, stability, and activity exist for the regulation of CtrA during cell cycle [9,10]. The phosphorylated form of CtrA (CtrA∼P) represents the active form that binds to DNA [11]. The phosphorylation of CtrA is catalyzed by an essential hybrid cell cycle histidine kinase/phosphatase, CckA, which phosphorylates CtrA through the single domain histidine phosphotransferase, ChpT (Fig. 1A and B) [12–15]. CckA gets autophosphorylated and it eventually transfers the phosphate group *via* ChpT to the master regulator, CtrA. In the swarmer, and pre-divisional cells, the kinase activity of CckA ensures the abundance of active CtrA∼P, while in the stalked cell compartment, the phosphatase activity of CckA is predominant ensuring the dephosphorylation, and degradation, of CtrA (Fig. 1A and B) [16]. The N-terminus of the CckA protein has two transmembrane helices and also contains two distinct sensory Per-ARNT-Sim domains, PAS-A and PAS-B [17,18]. The catalytic core of CckA comprises of a DHp (dimerization histidine phosphotransfer) domain, which is the site of histidine autophosphorylation, and an ATP binding catalytic assisting domain [19,20]. The C-terminal receiver domain in CckA shuttles the phosphate group to CtrA, through the ChpT

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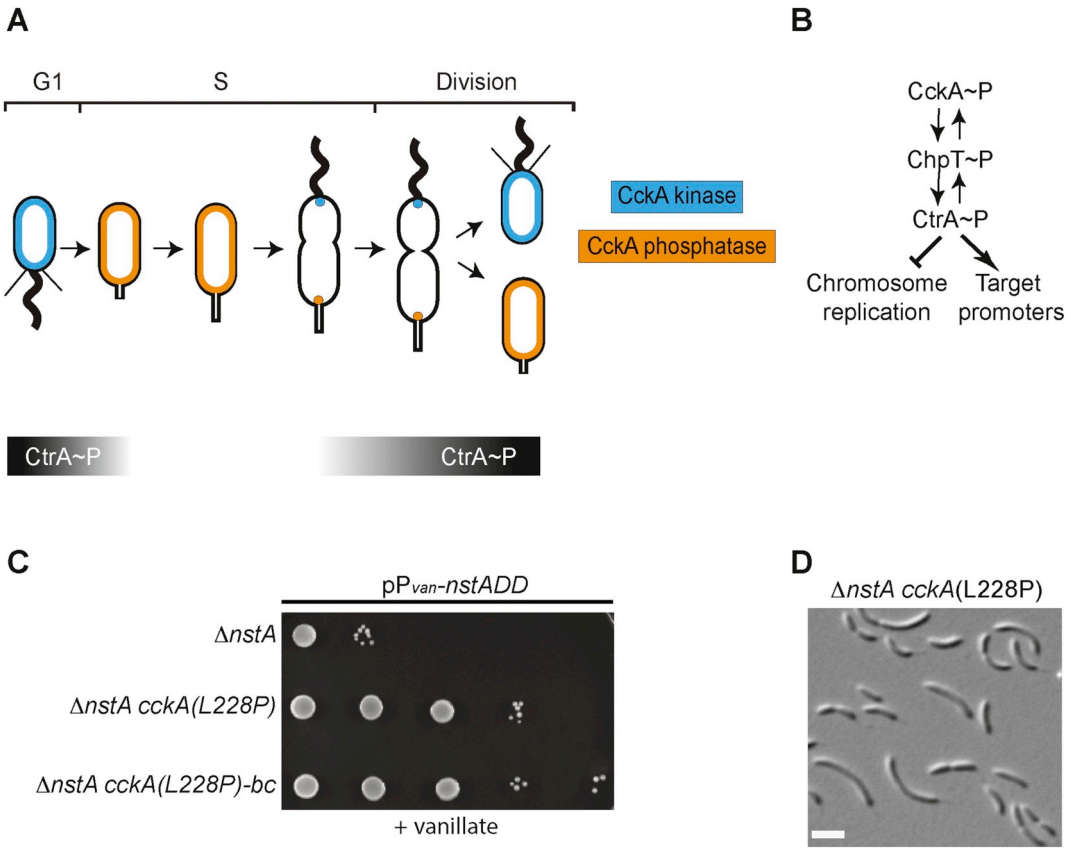

Fig. 1. Cell cycle regulation in *Caulobacter crescentus* by the CckA-CtrA pathway. (A) Schematic representation of the dual switching of CckA between the kinase mode (blue) and the phosphatase mode (orange) in the swarmer and the stalked cell compartments, respectively. The graded bars indicate the time during which CtrA (black) is present during the cell cycle. (B) The bidirectional flow of phosphate between CckA, ChpT and CtrA. In the swarmer cells, CckA transfers the phosphate group to the phosphotranferase, ChpT, which further donates the phosphate to CtrA. The active phosphorylated form of CtrA (CtrA∼P) can bind to various target promoters of several cell cycle regulated genes, as well as repress the initiation of chromosome replication. (C) Growth of Δ*nstA* cells overproducing NstADD, and harboring either *wild-type cckA* or *cckA*(L228P) or *cckA*(L228P) mutation back-crossed into a clean Δ*nstA* background [Δ*nstA cckA*(L228P)-*bc*]. Cells, as indicated, were diluted five folds and spotted on media containing 0.5 mM vanillate. (D) Differential interference contrast (DIC) image of the suppressor mutant, Δ*nstA cckA*(L228P). Scale bar: 2 μm.

phoshotransferase [21]. The PAS-A domain regulates density-dependent CckA kinase activity and its subcellular accumulation at the cell poles. The second PAS domain, PAS-B is needed for targeting CckA to the new cell pole and for cyclic-di-guanylate (*c*-di-GMP) stimulated CckA phosphatase activity [22–25].

Recent evidence have shown that in addition to developmental regulatory proteins such as CtrA, the cell cycle progression in *Caulobacter* is controlled by a cytoplasmic redox fluctuation [26]. We had shown that a redox-dependent regulator, NstA, whose activation is coupled to the cytoplasmic redox state, inhibits the DNA decatenation activity of topoisomerase IV (Topo IV) during the early stages of cell cycle [26]. Apart from the cytoplasmic redox control of NstA activity, additional layers of regulation for NstA exist at the level of transcription by the transcription factors, GcrA and CcrM, and at the level of protein abundance by the ClpXP protease. A stable version of NstA, NstADD, is resistant to protein degradation by ClpXP. Overproduction of NstADD from an inducible promoter induces lethality in *Caulobacter* [26]. In this study, we investigated the regulatory networks that possibly fine-tune NstA activity *in vivo*. Towards this, we exploited the lethality induced by NstADD, to conduct an unbiased forward genetic screen to analyze extragenic suppressor mutation(s) that can negate NstADD toxicity. Strikingly, through this screen, we have identified suppressor mutations in *cckA* that influences the DNA binding activity of CtrA in a distinctive manner. We show that the CckA(L228P) mutation enhances the CtrA∼P levels. Surprisingly, the increase in CtrA∼P levels does not result in an increase of CtrA binding to all CtrA binding regions on the chromosome. The DNA binding of CtrA is specifically increased only at the C_{ori} and a very small sub-set of CtrA dependent promoters. Finally, we show that the enhanced binding of CtrA to the C_{ori} rescues the toxicity caused by NstADD by possibly slowing down the chromosome replication process to compensate for the slowed down segregation caused by the inhibitory effects of NstADD on the Topo IV.

2. Materials and methods

2.1. Growth conditions and media

Caulobacter strains were grown on rich PYE media (0.2% peptone, 0.1% yeast extract, 1 mM $MgSO_4$, 0.5 mM $CaCl_2$) or M5G media (M5G low-phosphate medium: 10 mM PIPES, pH 7, 1 mM NaCl, 1 mM KCl, 0.05% NH_4Cl, 0.01 mM Fe/EDTA, 0.2% glucose, 0.5 mM $MgSO_4$, 0.5 mM $CaCl_2$ and 0.05 mM phosphate) [27,28], and incubated at 29 °C, unless specifically mentioned. The *Caulobacter* strains were subjected to electroporation, øCr30 mediated transductions, and intergeneric conjugations (using *E. coli S17-1*) as previously described [29–31]. *E. coli* strains, EC100D (Epicentre, WI, USA), and S17-1 were grown on LB media and incubated at 37 °C, unless specifically mentioned.

2.2. In vivo phosphorylation

In vivo phosphorylation experiments were performed as described previously [32]. Briefly, single colony of cells picked from a PYE agar