

A signal transduction protein cues proteolytic events critical to *Caulobacter* cell cycle progression

Dean Y. Hung and Lucy Shapiro*

Department of Developmental Biology, Beckman Center, Stanford University School of Medicine, Stanford, CA 94305

Contributed by Lucy Shapiro, August 16, 2002

Temporally controlled proteolysis of the essential response regulator, CtrA, is critical for cell cycle progression in *Caulobacter crescentus*. CtrA binds to and silences the origin of replication in swarmer cells. The initiation of replication depends on the proteolysis of CtrA. We present evidence that DivK, an essential single-domain response regulator, contributes to the control of the G₁-S transition by signaling the temporally controlled proteolysis of CtrA. In a *divK-cs* mutant at the restrictive temperature, the initiation of DNA replication is blocked because of the retention of CtrA. A shift of cells from restrictive to permissive temperature results in rapid degradation of CtrA, initiation of DNA replication, and the resumption of cell cycle progression, including the ordered expression of genes involved in chromosome replication and polar organelle biogenesis. CtrA binds to and regulates the promoters of two genes critical to its temporally controlled proteolysis, *divK* and *clpP*, providing a transcriptional feedback loop for the control of cell cycle progression.

C*aulobacter crescentus* undergoes asymmetric cell divisions that produce morphologically distinct daughter cells: a motile swarmer cell and a stalked cell. The stalked-cell progeny initiates DNA replication and assembles a flagellum at the pole opposite the stalk, yielding an asymmetric predivisional cell (see Fig. 1B). The progeny swarmer cell differentiates into a stalked cell and only then can replication begin. Thus, the timed biogenesis of polar structures is coordinated with DNA replication and cell division. The order of expression of these events is cued by two-component signal transduction proteins (1–4). Among these is an essential response regulator, CtrA (1), that is directly responsible for the transcriptional control of at least 55 cell cycle-regulated operons (5). In addition, CtrA~P binds to five sites in the origin of replication in swarmer cells, preventing the formation of the replisome (1). At the swarmer-to-stalked cell transition, CtrA is cleared from the cell by temporally controlled proteolysis (6) mediated by the ClpXP protease (7), which frees the origin for replication initiation. After the initiation of replication, CtrA proteolysis is halted, and a positive transcriptional feedback loop results in the accumulation of new CtrA (8), thus preventing premature reinitiation of DNA replication. As CtrA accumulates, it is converted to its active form by phosphorylation of an aspartate residue. Thus, multiple events control the temporal and spatial activity of CtrA, including proteolysis of CtrA at the G₁-S transition and in the stalked portion of the predivisional cell, and activation by phosphorylation. CtrA activation is the culmination of a poorly understood signal transduction network involving the histidine kinases CckA (4), PleC (9–11), DivJ (11, 12), DivL (13), and the essential single-domain response regulator, DivK (2). The CckA, PleC, and DivJ histidine kinases are dynamically localized to the cell poles, each at distinct stages of the cell cycle (4, 11), providing yet another level of control, integrating temporal and spatial signals.

Here, we focus on the contribution of the DivK response regulator to the control of cell cycle progression. Strains bearing a *divK* cold-sensitive (*cs*) mutation form filamentous cells with aberrant stalks (2). A suppressor of the *divK* mutant phenotype was previously mapped to a mutation in the DNA-binding

domain encoded by *ctrA*, providing a genetic link between DivK and CtrA (12). Unlike CtrA, the DivK protein is present and phosphorylated throughout the cell cycle, although it is dynamically localized to the cell poles at specific times in the cell cycle (14). The cytoplasmic DivK protein may transiently interact with other two-component signal transduction proteins known to colocalize at the cell poles. We present evidence here that at least one function of DivK is to regulate the proteolysis of CtrA at the G₁-S transition. In a *divK-cs* mutant at the restrictive temperature, the retention of CtrA prevents the initiation of DNA replication and cell cycle progression. This cell cycle block is reversible; on shift to permissive temperature, CtrA is proteolyzed and DNA replication is initiated. Release from the cell cycle block enables groups of temporally controlled genes to be transcribed in the order observed in wild-type cells. The cell cycle-regulated proteolysis of CtrA is part of a transcriptional feedback loop in which CtrA binds to and regulates the promoters of both the *divK* regulator and the *clpP* protease (5).

Materials and Methods

Construction of the *divK-cs* Strain. The original *divK-cs* mutant was identified in a screen to isolate cold-sensitive (*cs*) suppressors of a *pleC* temperature-sensitive mutant (10). Analysis of one of these suppressors, *divK341* (2), revealed that the mutation is a D90G change in a conserved response regulator residue (15). We constructed a version of this mutant in a CB15N background by PCR-based site-directed mutagenesis. The resulting strain, LS3570, contained the *divK-cs* mutation with all previously described phenotypes of *divK341*.

Synchronizations, Immunoblots, Immunoprecipitations, and Flow Cytometry. Cultures grown at 33°C in minimal M2G media were synchronized by isolating swarmer cells by using Ludox density centrifugation (1). Swarmer cells were incubated at either 20°C or 33°C, and aliquots were taken at selected times. Immunoblots (14) and immunoprecipitations (6) were performed as described. Samples for flow cytometry (14) were analyzed by using a Beckton Dickinson FACStar Plus machine. Data were collected and analyzed by using FLOJO software (Stanford University).

In Vivo Phosphorylation Assay. *In vivo* phosphorylation experiments were performed as described (14). M5G culture medium was supplemented with glutamate (1 mM). The cells were labeled for 3 min with 100 μCi [³²P]H₃PO₄ and samples were immunoprecipitated with anti-CtrA and anti-DivK sera (14). Radiolabeled precipitates were resolved on a SDS-15% polyacrylamide gel and visualized and quantified on a Molecular Dynamics PhosphorImager.

DNA Microarray Analysis. RNA preparation and hybridization to microarrays was performed as described (16). Reference RNA was from unsynchronized CB15N grown at 30°C. Scanning was performed on a GeneMachine, and spots determined by using

Abbreviations: FACS, fluorescence-activated cell sorter; *cs*, cold-sensitive.

*To whom reprint requests should be addressed. E-mail: shapiro@cngm.stanford.edu.

GENOPIX software. Each array was normalized, and genes represented more than once on the array were averaged. Three replicates were performed and the results for each gene ratio averaged. The initial 240-min time point was used as the denominator for each time point to determine the change in transcription levels relative to the 240-min time point.

Primer Extensions and DNaseI Footprints. Total RNA was isolated from wild-type CB15N cultures by hot phenol extraction. Primer extension reactions were carried by using the Superscript II Reverse Transcriptase (GIBCO/BRL) kit and the oligonucleotide primer 5'-TATCCTCCACGATGAGGACC-3' complementary to a region within the *divK* gene. Sequencing reactions were performed with the primer above, and resolved on the same gel. DNaseI-footprinting experiments were performed as described (17).

Results

A *divK-cs* Mutant Arrests at the G₁-S Transition. The *divK-cs* strain has a cell division defect and forms abnormally long stalks when grown at the restrictive temperature of 20°C (ref. 2; Fig. 1A). Fluorescence-activated cell sorter (FACS) assays of wild-type cells at 20°C showed the presence of two chromosomes, whereas *divK-cs* at 20°C showed a predominance of cells with a single chromosome, suggesting that on shift to the restrictive temperature, the *divK-cs* mutant was unable to initiate a new round of DNA replication. At the permissive temperature, *divK-cs* cells were similar to wild-type both in chromosome number and distribution of cell types.

To observe the morphological transitions of synchronized *divK-cs* cells at the restrictive temperature, swarmer cells were isolated from a culture grown at 33°C, then shifted to restrictive temperature. At the time of the shift, *divK-cs* swarmer cells were motile and seemed only slightly more filamentous than wild-type cells (Fig. 1B). These swarmer cells differentiated into stalked cells at approximately 120 min, whereas isogenic wild-type swarmer cells completed the swarmer-to-stalked cell transition at 90 min. After 240 min at 20°C, the mutant culture contained nonmotile, elongated stalked cells, whereas the wild-type culture had progressed to the predivisional cell stage. Thus, *divK-cs* swarmer cells at the restrictive temperature are able to complete the morphological transition to stalked cells. These stalked cells continue to increase in size, but are unable to divide.

To determine whether the elongated *divK-cs* stalked cells held at restrictive temperature for 240 min retain the ability to undergo cell division, we shifted these cells back to the permissive temperature, 33°C, and observed morphological changes. At 300 min, cells started to constrict at the division plane, and by 320 min, most cells had divided (Fig. 1B). Assays of *divK-cs* colony-forming units after a shift to the restrictive temperature showed that the number of viable cells remained almost constant for 6 h (Fig. 2A). After a shift back to permissive temperature at 240 min, the number of colony-forming units nearly doubled, consistent with the observed resumption in cell division.

Chromosome number was examined in synchronized populations of *divK-cs* cells by using FACS analysis (Fig. 2B). At the restrictive temperature, the majority of cells have stalks and have one chromosome at all time points, consistent with a block at the G₁-S transition. Although replication initiation occurs at the swarmer-to-stalked cell transition in wild-type cells and in *divK-cs* cells at the permissive temperature, the two events are uncoupled in *divK-cs* at the restrictive temperature. When a portion of the *divK-cs* culture incubated at the restrictive temperature was shifted back to the permissive temperature at 240 min, a rapid progression to cells with two chromosomes occurred, consistent with an increase in the number of viable cells (Fig. 2B). Thus, the G₁-S block is reversible, suggesting that functional DivK is required for the initiation of DNA replication.

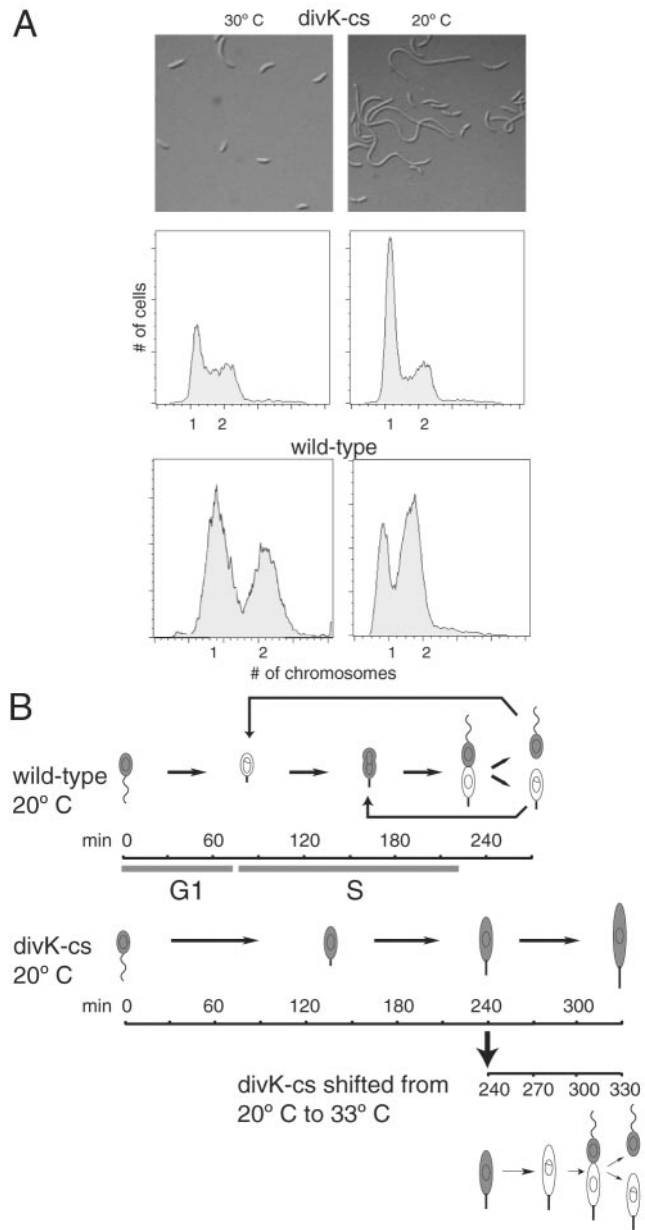


Fig. 1. A *divK-cs* mutant at the restrictive temperature blocks the G₁-S transition and accumulates elongated stalked cells. (A) Light microscopy of a *divK-cs* strain grown in rich media at the permissive temperature, 30°C, and at the restrictive temperature, 20°C. Below is the FACS analysis of the *divK-cs* mutant strain and wild-type *C. crescentus*. At the restrictive temperature, only the *divK-cs* cells accumulate single chromosomes. (B) Schematic of the morphology of wild-type and *divK-cs* synchronous cultures grown at the restrictive temperature (20°C) and *divK-cs* cells shifted to permissive temperature (33°C) at 240 min. Swarmer cells were isolated from cultures grown at 33°C and then incubated at 20°C in minimal media for 240 min. Wild-type cells differentiated into stalked cells at 90 min, and divided at 270 min, whereas *divK-cs* stalked cells appeared at 120 min and remained as elongated stalked cells. An aliquot of the *divK-cs* culture at 20°C shifted back to 33°C at 240 min completed division by 330 min. Shaded areas indicate presence of CtrA.

It was shown previously that transcription of genes involved in DNA replication occurs coincident with the onset of S-phase (16). To determine when these genes are transcribed in *divK-cs* cells shifted from the restrictive temperature back to the permissive temperature, we performed microarray analysis of total RNA (Fig. 3). After growth for 240 min at the restrictive

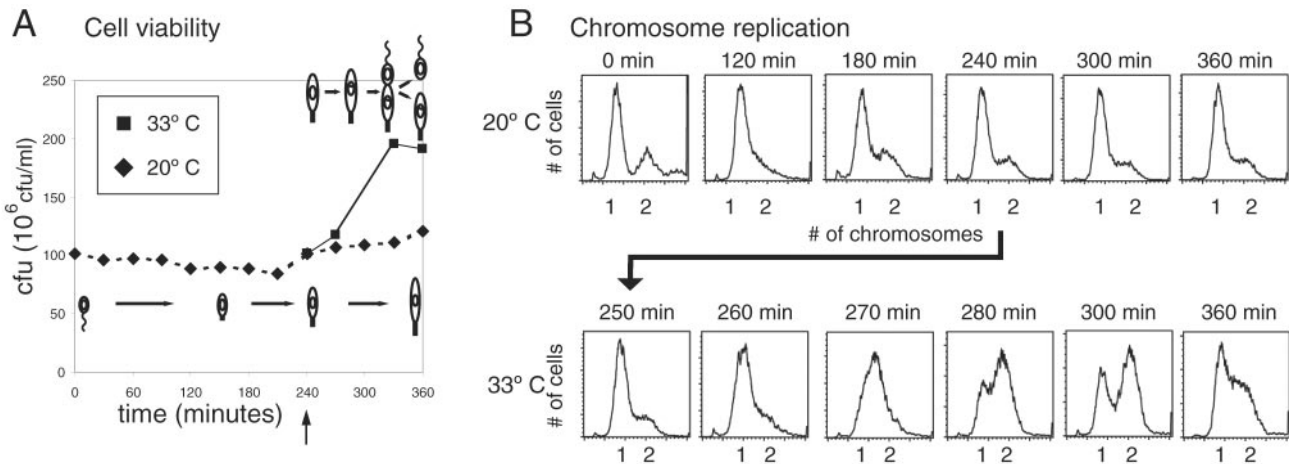


Fig. 2. *divK-cs* cultures shifted back to permissive temperature complete DNA replication and cell division. (A) At the restrictive temperature, *divK-cs* cultures had constant colony-forming units (cfu). On shift to the permissive temperature after 240 min at 20°C, the number of colony-forming units doubled, indicating the completion of cell division. (B) FACS analysis of synchronized *divK-cs* at 20°C and 33°C. At restrictive temperature, cells had predominantly one chromosome. After shift back to permissive temperature at 240 min, cells completed DNA replication by 300 min.

temperature, the expression of multiple genes involved in DNA replication is reduced relative to wild-type, but is induced 20 min after the shift back to the permissive temperature (Fig. 3). Because the synthesis of new DNA causes a shift to a two-

chromosome peak 20 min after the shift to the permissive temperature (Fig. 2B), these data suggest that the multiple steps in DNA replication are blocked in the *divK-cs* mutant at restrictive temperature and that this block is relieved on return to the permissive temperature.

In wild-type cells, the transcription of genes required for flagellar and pili biogenesis occurs after the onset of DNA replication gene expression (16). After the shift of *divK-cs* from restrictive back to permissive temperature, genes involved in flagella and pili biogenesis were turned on 20 min after the DNA replication genes (Fig. 3). Microarrays recapitulated the flagellar transcriptional cascade with class II genes expressed first, followed by class III and class IV genes. In a parallel manner, the transcriptional cascade of pili genes was initiated at 270 min. Thus, the signal transduction block in the *divK-cs* mutant halts the initiation of DNA replication, likely because of the retention of CtrA, and also blocks the expression of the DNA replication enzymes, but does not block stalk biogenesis. The block in the G₁-S transition results in the downstream block in flagellar and pili biogenesis, which is reversible only after DivK function is restored.

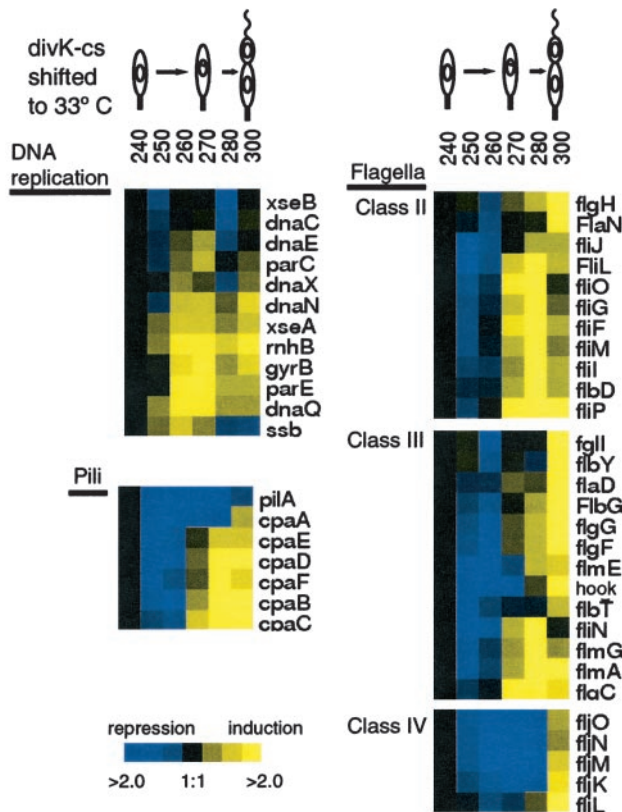


Fig. 3. Microarray analysis of total RNA collected at the indicated times after a shift of *divK-cs* from the restrictive temperature (20°C) back to the permissive temperature (33°C). The expression of genes involved in DNA replication, flagellar biogenesis, and pili biogenesis are shown. RNA was hybridized to microarrays containing 3,700 *C. crescentus* ORFs. The reference RNA was from a mixed population of cells grown at 33°C. Blue indicates a decrease and yellow indicates an increase relative to the 240-min time point. Black indicates no change. The data represent an average of three experimental repetitions.

CtrA Is Not Degraded in the Stalked Cell of a *divK-cs* Strain. To identify targets of the *divK* cell cycle function, we turned to the CtrA response regulator. CtrA regulates multiple transcriptional events during the *Caulobacter* cell cycle and it also functions to repress replication initiation by binding to the origin of replication (1, 19). At the G₁-S transition in wild-type cells, CtrA~P is cleared from the cell by targeted proteolysis, allowing the initiation of DNA replication (6, 7). An active, nonproteolyzable allele of CtrA causes an arrest at the G₁-S transition; cells stall with one chromosome and grow filamentous with long stalks (6), a phenotype parallel to that observed with the *divK-cs* mutant strain. To determine whether CtrA is retained after a shift of *divK-cs* cells from the permissive to restrictive temperature, we performed immunoblot analysis of CtrA levels in synchronized *divK-cs* cells held at the restrictive temperature (Fig. 4A). In wild-type cultures at 20°C, CtrA was present in swarmer, cleared from stalked cells at 90 min, and reappeared at the predivisional stage. In the *divK-cs* strain at 20°C, CtrA was present at all time points, suggesting that the observed block in the G₁-S transition is due to the continued repression of replication initiation by CtrA. Although CtrA is present in the G₁-blocked cells and these cells had differentiated into stalked cells, it could not be used to

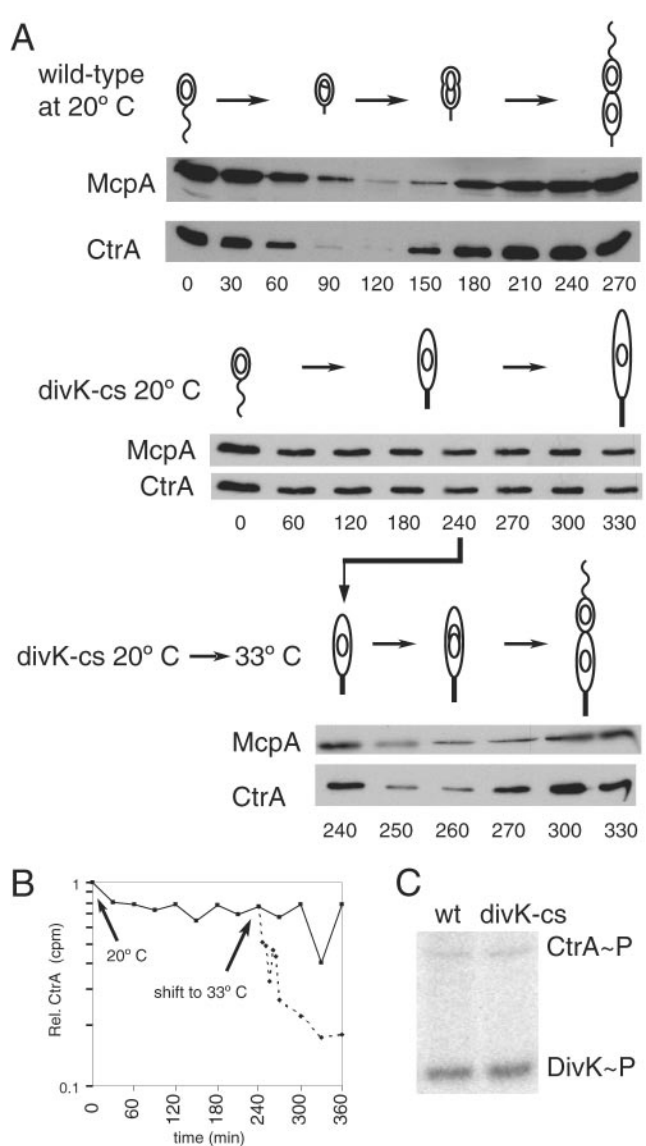


Fig. 4. (A) Turnover of the CtrA response regulator and the McpA chemoreceptor as a function of the cell cycle in wild-type (wt) and *divK-cs* cells grown at the restrictive temperature. CtrA is cleared from wild-type cells by 90 min and reappears by 150 min. McpA is also cleared from stalked cells but somewhat later than CtrA. In the synchronized *divK-cs* mutant at 20°C, both proteins are present through 330 min. After 240 min at 20°C, *divK-cs* cells shifted to the permissive temperature (33°C) exhibited turnover of both proteins within 10 min. Accumulation of these proteins resumed within 20 min. (B) CtrA protein is stabilized in a *divK-cs* mutant carrying *ctrA* under the control of a xylose-inducible promoter (pID42). The pID42/*divK-cs* strain was grown at 33°C in M2G minimal media and isolated swarmer cells pulsed for 7 min with [³⁵S]Met simultaneous with xylose induction (0.3%). Growth continued at 20°C and at 240 min a portion of the culture was shifted back to 33°C, immunoprecipitated with antibody to CtrA, and separated proteins were analyzed on a Molecular Dynamics PhosphorImager. (C) Wild-type and *divK-cs* cells at the same OD₆₆₀ of 0.3 were labeled with [³²P]H₃PO₄, and both DivK and CtrA proteins were immunoprecipitated with their respective antisera. Phosphorylation was visualized on a Molecular Dynamics PhosphorImager. CtrA~P and DivK~P are present at comparable levels in both the *divK-cs* mutant and wild-type.

initiate the flagellar transcriptional cascade (Fig. 3). These results suggest that additional events, possibly mediated by DivK, coordinate the activation of genes by CtrA~P. On shift back to permissive temperature after 240 min, CtrA

levels decreased rapidly (Fig. 4A). A sharp reduction in protein levels was apparent within 10 min, and this low level persisted until 270 min, when levels increased. The window of time with low levels of CtrA likely accounts for the release of CtrA from the origin and the consequent initiation of DNA replication.

Because a suppressor of *divK-cs* mapped to the *ctrA* gene (12), a possible explanation for the retention of CtrA in the stalked cell is that the mutant DivK protein at restrictive temperature binds inappropriately to CtrA, thereby preventing its proteolysis. At the permissive temperature, the release of the mutant protein would result in the observed rapid onset of CtrA proteolysis. To test this possibility, we examined the cell cycle abundance of another protein, the McpA chemoreceptor, that is normally degraded in the stalked cell (18) (Fig. 4A). In synchronized *divK-cs* cells at restrictive temperature, McpA was continuously present. On a shift back to the permissive temperature, we observed a rapid onset of McpA proteolysis demonstrating that two independent proteolytic events, CtrA and McpA degradation, are blocked when DivK-cs is inactivated, arguing against a CtrA-specific binding of the DivK-cs mutant protein at the restrictive temperature. Thus, DivK may signal the activation of a factor that facilitates proteolysis of both of these proteins in stalked cells.

CtrA Is Stabilized in *divK-cs* Mutant Cells at the Restrictive Temperature. To determine the half-life of CtrA in a *divK-cs* mutant at the restrictive temperature, we moved a xylose-inducible copy of *ctrA* on a plasmid (pID42) into the *divK-cs* strain. Swarmer cells were isolated from this strain grown at 33°C and then shifted to the restrictive temperature (20°C). On shift to 20°C, expression of the plasmid-borne *ctrA* was induced with xylose, pulse-labeled with [³⁵S]methionine, then chased with excess unlabeled methionine and casamino acids. Cell samples were taken at sequential time points. At 240 min, a portion of the culture was shifted back to 33°C, and samples were taken from both cultures until 360 min. Extracts from each sample were immunoprecipitated with anti-CtrA antibody. Relative levels of ³⁵S-labeled CtrA were determined (Fig. 4B). CtrA levels remained constant in cells held at the restrictive temperature, but on shift to the permissive temperature, the half-life of CtrA dropped to approximately 5 min, consistent with values obtained in wild-type stalked cells (6). Thus, CtrA is stable in *divK-cs* mutant cells held at 20°C for up to 360 min. The shift back to the permissive temperature resulted in a near-wild-type rate of CtrA degradation, suggesting that the *divK-cs* mutant phenotype is rapidly reversible with respect to CtrA proteolysis.

The transcription of *ctrA* is normally cell cycle-controlled; transcription is induced after the initiation of DNA replication in the early predivisional cell (1, 8). To determine whether *ctrA* transcription is constitutive in the *divK-cs* mutant, contributing to the apparent retention of CtrA in stalked cells, we moved a plasmid containing the full *ctrA* promoter driving β-galactosidase (LS2882) into the *divK-cs* strain. Immunoprecipitation levels showed that, as in the wild-type case, CtrA transcription was cell cycle-controlled (data not shown). Thus, inappropriate transcription of *ctrA* in synchronized *divK-cs* cells held at the restrictive temperature does not contribute to the accumulation of CtrA protein.

The DivK-cs Protein Is Competent for Phosphorylation. If DivK signals the activation of the ClpXP degradation of CtrA, a possible explanation for the *divK-cs* phenotype is that the mutation affects the phosphorylation of DivK-cs protein. In wild-type cells, DivK is phosphorylated throughout the cell cycle (14). To determine whether the DivK-cs protein is phosphorylated at the restrictive temperature, we labeled *divK-cs* cells with [³²P]H₃PO₄, and immunoprecipitated DivK-cs protein with anti-DivK antibody (Fig. 4C). DivK~P levels were comparable in

present at the swarmer-to-stalked cell transition (6). Assays of CtrA-turnover rates in the *divK-cs* mutant at the restrictive temperature revealed that CtrA is stabilized, suggesting that replication initiation is blocked because of CtrA-mediated repression of replisome formation. Despite the G₁-S arrest, other cellular processes such as growth and biogenesis of the stalk are not blocked, indicating that, in the absence of DivK function, the coordination of cell cycle events is disrupted.

DivK signaling is likely to affect multiple proteolytic events at the G₁-S transition because in addition to CtrA, the McpA chemoreceptor, another protein degraded at this time in the cell cycle, is stabilized in the *divK-cs* mutant. The stall at the G₁-S transition is released rapidly on a shift to permissive temperature and is coincident with CtrA proteolysis, supporting the hypothesis that continued presence of CtrA caused the observed block in cell cycle progression. Release from the G₁-S stall is accompanied by the expression of genes involved in the initiation of DNA replication, which is followed by the ordered expression of genes involved in the biogenesis of the polar flagellum and pili, and the normal resumption of cell cycle progression.

There are several possible mechanisms that would explain the malfunction of DivK-cs: (i) A defect in phosphotransfer ability of DivK-cs at the restrictive temperature prevents the activation of a protein involved in proteolytic regulation; (ii) the DivK-cs protein has a structural defect, preventing proper binding to another factor necessary to trigger the proteolytic machinery; (iii) wild-type DivK is itself the temporal specificity factor for CtrA degradation and works by binding directly to CtrA, thereby causing it to become a target for proteolysis. Several lines of evidence argue against the last possibility. First, shifting synchronized *divK-cs* cells held at restrictive temperature (20°C)

back to permissive temperature (33°C) caused an immediate decline in CtrA protein levels in less than 5 min, suggesting that activation of CtrA proteolysis is likely not due to new synthesis of DivK-cs protein at the permissive temperature, but that the DivK-cs protein already present at the time of shift goes through a reversible change to a more active form, thus enabling the proteolysis of CtrA. Second, the degradation of McpA and CtrA after shift to permissive temperature in synchronized *divK-cs* cells suggests that DivK does not specifically target CtrA for proteolysis by ClpXP, but acts in a regulatory step upstream of CtrA and McpA proteolysis. Finally, an *in vitro* experiment using active ClpXP and purified DivK~P did not result in CtrA degradation (K. Ryan, personal communication). Thus, we propose that the DivK response regulator plays a critical role in the signal transduction pathway that cues the temporally regulated proteolysis of CtrA. This event, in turn, allows the completion of the G₁-S transition and cell cycle progression.

The observation that the *divK* promoter is activated by CtrA~P reveals a regulatory loop in which CtrA controls expression of a gene whose product signals CtrA proteolysis (Fig. 5D). It has recently been demonstrated that CtrA also binds to the *clpP* promoter and regulates the expression of the ClpP component of the protease that is responsible for CtrA turnover (5). Thus, signaling through DivK is one component of a complex regulatory feedback network that controls the coordination of cell cycle events by the CtrA response regulator.

We thank Alison Hottes and Swaine Chen for help in analysis of microarray data. We thank members of the Shapiro laboratory for critical reading of the manuscript. This work was supported by National Institutes of Health Grant 32506/5120M2 and Defense Advanced Research Planning Agency Grant MDA972-00-0032.

- Quon, K. C., Marczyński, G. T. & Shapiro, L. (1996) *Cell* **84**, 83–93.
- Hecht, G. B., Lane, T., Ohta, N., Sommer, J. M. & Newton, A. (1995) *EMBO J.* **14**, 3915–3924.
- Jenal, U. (2000) *FEMS Microbiol. Rev.* **24**, 177–191.
- Jacobs, C., Domian, I. J., Maddock, J. R. & Shapiro, L. (1999) *Cell* **97**, 111–120.
- Laub, M. T., Chen, S. L., Shapiro, L. & McAdams, H. H. (2002) *Proc. Natl. Acad. Sci. USA* **99**, 4632–4637.
- Domian, I. J., Quon, K. C. & Shapiro, L. (1997) *Cell* **90**, 415–424.
- Jenal, U. & Fuchs, T. (1998) *EMBO J.* **19**, 5658–5669.
- Domian, I. J., Reisenauer, A. & Shapiro, L. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 6648–6653.
- Wang, S. P., Sharma, P. L., Schoenlein, P. V. & Ely, B. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 630–634.
- Sommer, J. M. & Newton, A. (1991) *Genetics* **129**, 623–630.
- Wheeler, R. T. & Shapiro, L. (1999) *Mol. Cell* **4**, 683–694.
- Wu, J., Ohta, N. & Newton, A. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 1443–1448.
- Wu, J., Ohta, N., Zhao, J. L. & Newton, A. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 13068–13073.
- Jacobs, C., Hung, D. & Shapiro, L. (2001) *Proc. Natl. Acad. Sci. USA* **98**, 4095–4100.
- Hecht, G. B. (1995) Ph.D. thesis (Princeton Univ., Princeton, NJ).
- Laub, M. T., McAdams, H. H., Feldblyum, T., Fraser, C. M. & Shapiro, L. (2000) *Science* **290**, 2144–2148.
- Reisenauer, A., Quon, K. & Shapiro, L. (1999) *J. Bacteriol.* **181**, 2430–2439.
- Alley, M. R., Maddock, J. R. & Shapiro, L. (1993) *Science* **259**, 1754–1757.
- Quon, K. C., Yang, B., Domian, I. J., Shapiro, L. & Marczyński, G. T. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 120–125.
- Aldridge, P. & Jenal, U. (1999) *Mol. Microbiol.* **32**, 379–391.