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CtrA mediates a DNA replication checkpoint that prevents cell division in *Caulobacter crescentus*

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Coordination of DNA replication and cell division is essential in order to ensure that progeny cells inherit a full copy of the genome. *Caulobacter crescentus* divides asymmetrically to produce a non-replicating swarmer cell and a replicating stalked cell. The global response regulator CtrA coordinates DNA replication and cell division by repressing replication initiation and transcription of the early cell division gene *ftsZ* in swarmer cells. We show that CtrA also mediates a DNA replication checkpoint of cell division by regulating the late cell division genes *ftsQ* and *ftsA*. CtrA activates transcription of the P_{QA} promoter that co-transcribes *ftsQA*, thus regulating the ordered expression of early and late cell division proteins. Cells inhibited for DNA replication are unable to complete cell division. We show that CtrA is not synthesized in pre-divisional cells in which replication has been inhibited, preventing the transcription of P_{QA} and cell division. Replication inhibition prevents the activation of the *ctrA* P2 promoter, which normally depends on CtrA phosphorylation. This suggests the possibility that CtrA phosphorylation may be affected by replication inhibition.

Keywords: *Caulobacter*/cell cycle/cell division/checkpoint/CtrA

Introduction

In all organisms, the coordination of DNA replication and cell division is important for optimal viability. Cells in which replication and segregation proceed normally possess control mechanisms that regulate the order of cell cycle stages. In addition, both prokaryotic and eukaryotic organisms have elaborated intricate checkpoint mechanisms to inhibit cell division when DNA is damaged, when replication is stopped or delayed, or when chromosome segregation is defective. In eukaryotes, checkpoint mechanisms ensure that mitosis only occurs if DNA replication has been completed and prevent mitosis when DNA is damaged (Weinert, 1998).

One example of a prokaryotic checkpoint is the RecA-dependent induction of the SOS response that inhibits cell division in the presence of DNA damage in *Escherichia coli*. The SOS-induced SulA protein inhibits cell division by preventing the polymerization of the cell division initiation protein FtsZ (Bi and Lutkenhaus, 1993). Differentiating bacteria also couple cell differentiation to progression through the cell cycle. For example, *Bacillus subtilis* utilizes a checkpoint to coordinate DNA replication and sporulation if replication is inhibited, by blocking the phosphorelay pathway that normally activates the sporulation transcription factor SpoOA (Ireton and Grossman, 1992, 1994). As a consequence, cells are unable to activate sporulation genes. Thus, in both eukaryotes and prokaryotes, growth and the correct execution of developmental programs require that multiple individual events proceed in an orderly fashion. How is the timing of individual cell cycle and developmental events regulated such that they occur in the proper order?

The asymmetric cell division of *Caulobacter crescentus* exemplifies the complexity of the regulatory mechanisms that coordinate the progression through the cell division cycle with developmental events (Figure 1). Each cell division yields two different progeny cells: a motile, non-replicating swarmer cell and a replication-competent, sessile stalked cell (Stove and Stanier, 1962; Brun *et al*., 1994). The asymmetric pre-divisional cell has a flagellum at one pole and a stalk and holdfast at the opposite pole. The stalked cell re-enters S phase immediately after cell division and grows into an asymmetric pre-divisional cell. In contrast, the swarmer cell must differentiate into a stalked cell before entering S phase. The establishment of cellular asymmetry prior to cell division is tightly coupled to progression through the cell cycle by various checkpoints. For example, the development of the flagellated pole into a stalked pole is not simply coupled to cell mass increase but requires the initiation of cell division (Ohta and Newton, 1996; Ohta *et al*., 2000). In turn, DNA replication and cell division are regulated such that they occur at specific stages during the developmental program. Furthermore, disruption of DNA replication inhibits many temporally controlled events including synthesis of the CcrM DNA methyltransferase (Stephens *et al*., 1995b) and flagellum biosynthesis (Dingwall *et al*., 1992; Stephens and Shapiro, 1993). Cell division is also inhibited in the absence of DNA replication, indicating that there is a checkpoint connecting cell division to chromosome replication (Degnen and Newton, 1972a,b; Osley and Newton, 1977, 1980; Ohta *et al*., 1990).

It has been shown recently that the *ftsZ*, *ftsQ* and *ftsA* cell division genes of *Caulobacter* are transcribed in an order that reflects their order of action in cell division (Kelly *et al*., 1998; Sackett *et al*., 1998) (Figure 1). Transcription of *ftsZ* begins early in the cell cycle at the same time as the initiation of DNA replication (Kelly *et al*., 1998). The transcription of *ftsZ* is repressed in swarmer cells by the response regulator CtrA. CtrA also inhibits the initiation of DNA replication by binding to the origin of replication and repressing the strong origin promoter, P_S (Marczynski *et al*., 1995; Quon *et al*., 1998). The presence

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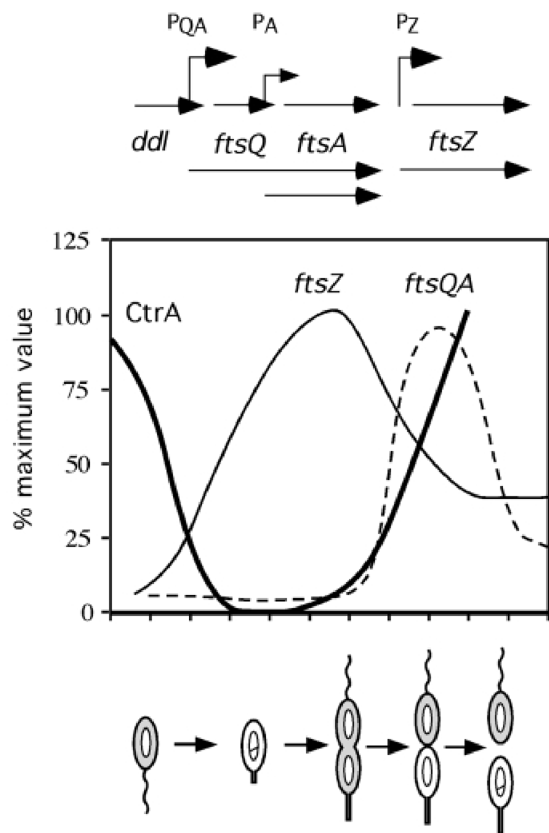

Fig. 1. Cell cycle rates of *ftsZ* and *ftsQA* transcription and CtrA concentration. The genetic organization and promoters of the *ddl–ftsQAZ* region are shown at the top. Coding regions are represented by arrows above gene names, transcriptional units by arrows below gene names, and promoters by bent arrows. A transcriptional terminator uncouples the transcription of *ftsQA* and *ftsZ*. The cell cycle transcription rates of *ftsZ* and *ftsQA* are shown in graph form, overlaid with the amount of CtrA present during the cell cycle (Kelly *et al*., 1998; Sackett *et al*., 1998). *ftsZ* transcription is at its maximum when the concentration of CtrA is at its minimum. *ftsQA* transcription increases in pre-divisional cells as the level of CtrA increases. A schematic of the *Caulobacter* cell cycle is shown, with internal circles representing non-replicating chromosomes, θ structures representing replicating chromosomes, and shading indicating the presence of CtrA. CtrA is present in swarmer cells and degraded during swarmer to stalked cell differentiation. CtrA reappears in pre-divisional cells and is degraded only in the stalked cell compartment just prior to division. Stalks are represented by thick lines and flagella by thin, curving lines.

and activity of CtrA are regulated by transcriptional control, phosphorylation and proteolysis (Domian *et al*., 1997, 1999; Quon *et al*., 1998). CtrA-P is present in swarmer cells and is degraded during swarmer to stalked cell differentiation by the ClpXP protease (Jenal and Fuchs, 1998). *ctrA* transcription is regulated by the opposite action of CtrA on its two promoters, repression of the weak promoter P1 and activation of the strong promoter P2 (Domian *et al*., 1999). The increased concentration of CtrA-P at the end of S phase represses *ftsZ* transcription (Kelly *et al*., 1998). Late in the cell cycle, just prior to cell separation, CtrA is degraded in the stalked cell compartment of the pre-divisional cell, allowing DNA replication initiation and transcription of *ftsZ* to start immediately in the stalked cell (Kelly *et al*., 1998; Domian *et al*., 1999). In addition to controlling the initiation of DNA replication and *ftsZ* transcription, CtrA-P initiates the flagellar regulatory hierarchy by activating the transcription of *fliQR*, *fliLM* and *fliF* (Quon *et al*., 1996) and activates the transcription of the *ccrM* DNA methyltransferase gene (Quon *et al*., 1996; Reisenauer *et al*., 1999).

The cell cycle control of cell division genes also extends to cell division genes that are required for late stages in division. The *ftsQ* and *ftsA* genes, which are required for late stages of cell division, are co-transcribed from promoter P_{QA} at the end of S phase when *ftsZ* transcription is repressed by CtrA (Sackett *et al*., 1998). This suggested the possibility that coupling of P_{QA} transcription to DNA replication could provide a checkpoint to link the transcription of late cell division genes to DNA replication (Sackett *et al*., 1998). Here we describe a novel replication checkpoint mechanism that prevents transcription of late cell division genes in the pre-divisional cell. We show that transcription of *ftsQ* and *ftsA* from the P_{QA} promoter requires DNA replication. These regulatory effects still occur in a *recA*⁻ strain and are thus not mediated by induction of the SOS response. These results suggest that coupling of DNA replication and cell division in *Caulobacter* may occur, at least in part, through regulation of P_{QA} transcription. We show that CtrA is a transcriptional activator of P_{QA}. CtrA is required *in vivo* for P_{QA} transcription, and CtrA-P binds directly to a CtrA recognition sequence in the P_{QA} promoter. Deletion or mutagenesis of this sequence abolishes promoter activity and CtrA binding. Transcription of *ftsZ*, which is normally repressed by CtrA, increases when DNA replication is inhibited. Immunoblot and immunoprecipitation analyses indicate that CtrA does not accumulate substantially in pre-divisional cells when DNA replication is inhibited. Under these conditions, the *ctrA* P2 promoter is inactive, suggesting that CtrA-P does not accumulate sufficiently or is sequestered. These results indicate that CtrA directly mediates the coupling of developmental gene expression and a late stage of cell division to DNA replication.

Results

DNA replication is required for ftsQA transcription

The transcription of P_{QA} begins during the end of the DNA replication cycle and peaks after the completion of DNA replication (Sackett *et al*., 1998) (Figure 1), suggesting that late transcription of *ftsQ* and *ftsA* could provide a checkpoint between DNA replication and cell division. To test this hypothesis, we inhibited DNA replication with hydroxyurea and monitored the transcription from the P_{QA} promoter using a fusion to a promoterless *lacZ* gene in the plasmid pMSP8LC. The concentration of hydroxyurea used does not affect RNA or protein synthesis (Dingwall *et al*., 1992; Stephens and Shapiro, 1993; Stephens *et al*., 1995a). Transcription was measured by pulse labeling proteins with [^{35}S]methionine and subsequently immunoprecipitating and quantitating labeled β-galactosidase protein; DNA replication was monitored by pulse labeling cells with [8-^{3}H]dGTP. DNA replication was reduced by 75% within 30 min of hydroxyurea treatment. After inhibition of DNA replication, transcription from the P_{QA} promoter decreased to 25% of the initial level within the first 30 min of hydroxyurea treatment (Figure 2A). As a negative control for our experimental conditions, we

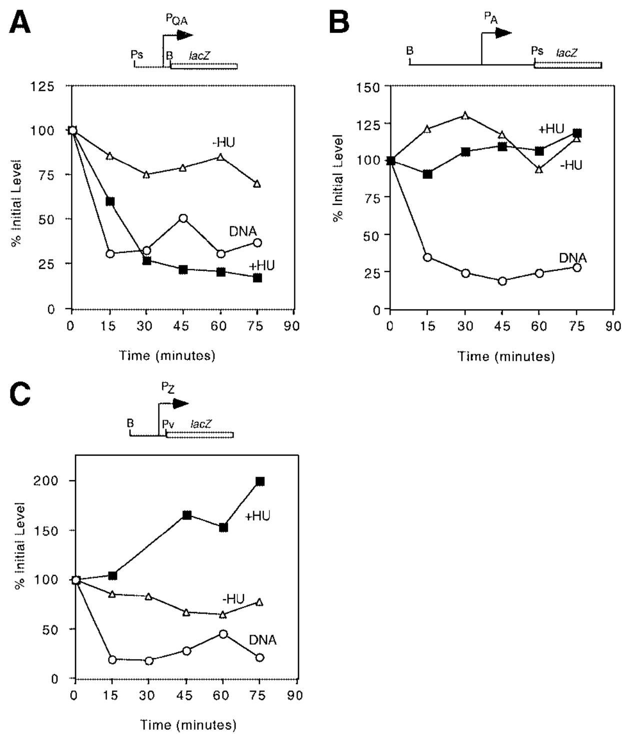

Fig. 2. Effect of DNA replication inhibition on transcription from the cell division promoters P_{QA}, P_A and P_Z. DNA synthesis was inhibited with hydroxyurea and the effects on transcription of the (**A**) P_{QA}, (**B**) P_A and (**C**) P_Z promoters fused to a promoterless *lacZ* were analyzed. Mixed cultures were divided into two samples: one treated with hydroxyurea (+HU) and one untreated sample (–HU). Cells were pulsed at 15 min intervals with [^{35}S]methionine, and β-galactosidase was immunoprecipitated. The level of labeled β-galactosidase was measured by phosphorimaging quantitation. DNA synthesis was analyzed, in the sample treated with hydroxyurea, by pulsing an aliquot with [8-^{3}H]dGTP and measuring the amount of labeled DNA. The diagram above each graph indicates the promoter fusion utilized.

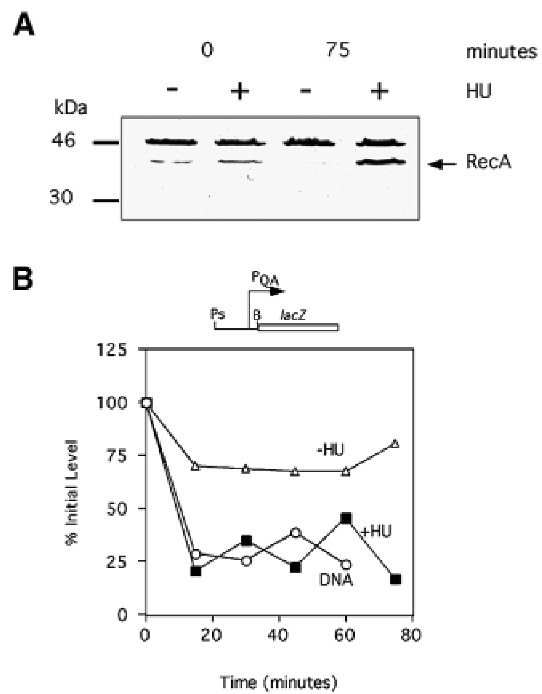

Fig. 3. Inhibition of expression of the P_{QA} promoter by the inhibition of DNA replication is not mediated by the SOS response. (**A**) DNA synthesis in wild-type strain NA1000 was inhibited with hydroxyurea (HU). RecA (arrow) concentration was measured by immunoblotting of samples before (0 min) and after (75 min) the addition of HU. (**B**) Expression from the P_{QA} promoter from plasmid pMSP8LC in the absence (+HU) or presence (–HU) of DNA replication in *recA*⁻ strain CM5256. DNA synthesis levels in the presence of hydroxyurea are denoted by 'DNA'.

measured the effect of replication inhibition on transcription from the P_A promoter in plac290/AJK2. In contrast to P_{QA}, transcription from the P_A promoter was not inhibited by hydroxyurea (Figure 2B). Transcription of P_A remained at approximately the same level as the untreated sample throughout the experiment. These results indicate that the decrease of transcription of *ftsQA* from the P_{QA} promoter is a specific response to DNA replication inhibition.

The DNA replication checkpoint for P_{QA} transcription is independent of RecA

Caulobacter has a UV-inducible *rec*-requiring DNA repair system analogous to the *E.coli* SOS response (Bender, 1984) that provides a possible link between DNA replication and transcription of the *ftsQA* genes. We first tested whether the addition of hydroxyurea to cultures of wild-type strain NA1000 resulted in the induction of the SOS response by monitoring RecA concentration with immunoblotting. Figure 3A illustrates that 75 min after addition of hydroxyurea, the concentration of RecA had increased substantially. To test if the SOS response was responsible for the inhibition of P_{QA} transcription in the absence of DNA replication, we analyzed the transcription of the P_{QA} promoter in a *recA*⁻ strain background CM5256 (O'Neill *et al.*, 1985). Transcription from the P_{QA} promoter was still inhibited upon inhibition of DNA synthesis in the *recA*⁻ strain (Figure 3B). This indicates that the effect of inhibiting DNA replication on transcription of P_{QA} is not due to induction of the SOS response.

Mutational analysis of P_{QA}

To begin to investigate the mechanisms that couple P_{QA} transcription to the cell cycle and DNA replication, we made 5′ and 3′ promoter deletion series to locate the P_{QA} regulatory sequences more precisely. The P_{QA} promoter previously was shown to be located in a 493 bp *Pst*I–*Bam*HI fragment (Sackett *et al.*, 1998) (Figure 4A). The 5′ deletion series showed that deletions of 298 and 333 bp from the *Pst*I site (pPQA-123LC and pPQA-88LC) had little impact on the amount of transcription (Figure 4B). The pPQA-123LC and pPQA-88LC fusions gave ~1600 Miller units of β-galactosidase activity compared with ~1900 for pPQA-421LC. A further deletion of 99 bp (pPQA+11LC) had a dramatic effect, lowering the activity to 19 Miller units. This indicates that essential promoter elements are located between –88 and +11.

The location of the P_{QA} promoter was delineated further by analyzing a 3′ promoter deletion series (Figure 4B). The longest construct, 5′ deletion series member pPQA-421LC, has a similar 5′ end point (6 bp difference) to the rest of the 3′ deletion series and a β-galactosidase activity of 1934 Miller units. A deletion of 822 bp from the 3′ end of pPQA-421LC, to make p3′QA+52, increased the β-galactosidase activity to 4983 Miller units. A deletion of 45 bp from 3′ deletion p3′QA+52, to make p3′QA+7,

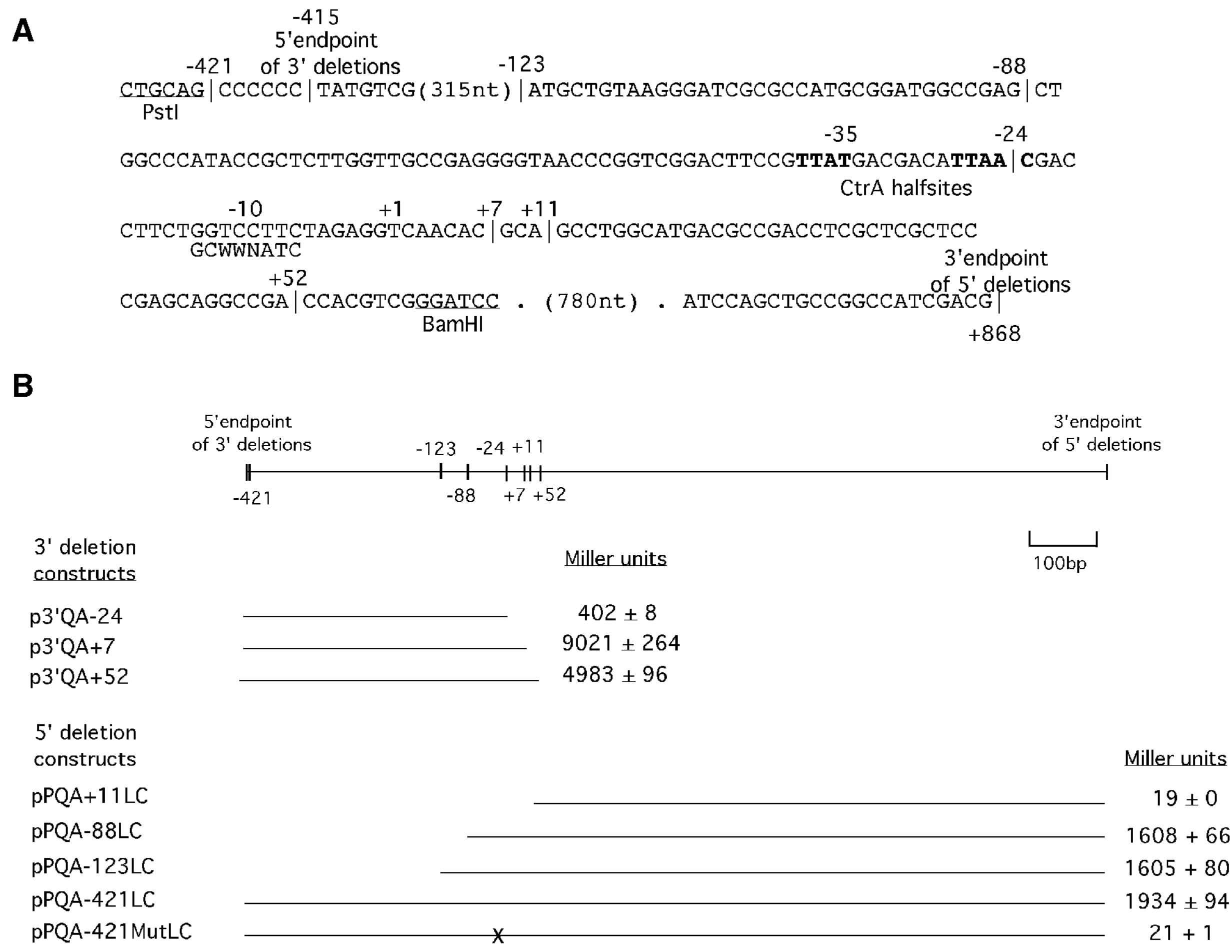

Fig. 4. Mutational analysis of the *ftsQA* promoter region. Transcriptional fusions of P_{QA} deletions to a promoterless *lacZ* gene were assayed for promoter activity. (**A**) A partial sequence of the P_{QA} region is shown with deletions marked. Deletions –24, +7 and +52 are a 3′ deletion series with a common 5′ end point at –415. Deletions –421, –123, –88 and +11 are a 5′ deletion series with a common 3′ end point at + 868. The CtrA-binding site is shown in bold and the –10 consensus sequence is shown. (**B**) Deletion fusion fragments are shown to scale, with the activity in Miller units indicated. pPQA-421MutLC is identical to pPQA-421LC except that the CtrA-binding site was changed from TTAT-N7-TTAAC to TTAT-N7-CGGCC. This mutation is shown as an 'X' on the pPQA-421MutLC plasmid. The β-galactosidase activity of each fusion is shown in Miller units with the background activity of the vector subtracted. Each value is the average of at least three assays, and the standard deviation is shown. The +1 coordinate was assigned arbitrarily based on the consensus of CtrA-activated promoters.

increased the amount of transcription to 9021 Miller units. A further deletion of 31 bp to form p3′QA-24 decreased the amount of transcription to 402 Miller units. The results of the 3′ deletions indicate that essential promoter elements are located between –24 and +7.

Together, the results of the promoter deletion experiments indicate that essential promoter elements are located downstream of –24 and upstream of +11. This region contains sequences similar to those of promoters that are positively regulated by the response regulator CtrA (Figure 4A) (Wu *et al*., 1998). In these promoters, CtrA binds to a region centered around –30 in relation to the transcription start site. The promoter region delineated by deletion analysis contains a putative CtrA-binding site (TTAT-N7-TTAAC) with only one deviation from the consensus sequence (TTAA-N7-TTAAC) and an appropriately spaced –10 region (4/7 matches). This promoter and regulatory sequence assignment is supported by the role of CtrA in the regulation of P_{QA} demonstrated below and disagrees with a previous tentative assignment of a promoter region based on primer extension (Sackett *et al*., 1998). Three 5′ ends were detected in the *ftsQ* mRNA (Sackett *et al*., 1998). Two of the 5′ ends were in regions devoid of promoter activity, indicating that the *ftsQA* mRNA is subject to processing (Sackett *et al*., 1998). Because there is clearly a promoter located between –24 and +7 that is ~50 nucleotides upstream of the other *ftsQ* mRNA 5′ end identified by primer extension (Sackett *et al*., 1998), we suspect that this 5′ end is also the product of a processing reaction.

CtrA is an activator of P_{QA} transcription

To determine if CtrA is an activator of P_{QA} transcription, we analyzed the transcription from the P_{QA} promoter on plasmid pMSP8LC in a strain containing a temperature-sensitive allele of *ctrA*, *ctrA401*. We compared the transcription of promoters P_{QA} which contains a putative CtrA recognition sequence, and P_A which does not contain a putative CtrA-binding site. At the permissive temperature, both promoter fusions produced an increase in β-galactosidase activity that was proportional to the increase in cell mass (Figure 5). At the non-permissive

temperature, the β-galactosidase activity from the P_{QA} promoter fusion decreased by ~50% within 150 min, the time required for approximately one mass doubling. This suggests that little transcriptional activity remained once CtrA was inactivated. In contrast, the β-galactosidase activity from the P_A promoter remained unaffected by the inactivation of CtrA (Figure 5).

The importance of the putative CtrA-binding site for P_{QA} promoter activity was determined by mutagenizing it from TTAT-N7-TTAAC to TTAT-N7-CGGCC. When fused to *lacZ* (pPQAMut-421LC), the mutagenized promoter resulted in only 21 Miller units of activity compared with the 1934 Miller units produced by the wild-type promoter in plasmid pPQA-421LC (Figure 4). To determine if CtrA binds to the putative CtrA-binding site, DNase I footprinting was performed on wild-type P_{QA} as well as the mutant CtrA-binding site. A 198 bp fragment from –141 to +57 containing the wild-type CtrA-binding site or the mutagenized CtrA-binding site was amplified by PCR using a ^{32}P-labeled 3′ primer and an unlabeled primer. His_6-CtrA-P protected a 22 nucleotide region from –17 to –38 on the wild-type promoter that also includes the CtrA-binding site (Figure 6). In contrast, His_6-CtrA-P failed to protect the mutagenized CtrA-binding site. These results, coupled with the absence of β-galactosidase activity in pPQA-421MutLC (Figure 4) and the *in vivo* requirement of CtrA for P_{QA} transcription (Figure 5), indicate that CtrA is an activator of P_{QA}.

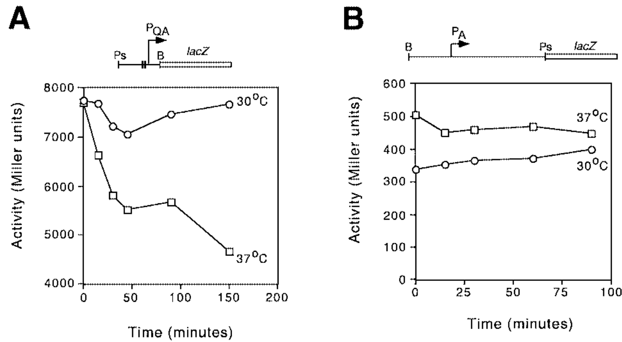

Fig. 5. CtrA is required for the transcription of the P_{QA} promoter, but not P_A. The β-galactosidase activity of (**A**) P_{QA} (pMSP8LC) and (**B**) P_A (plac290/AJK2) was monitored at the permissive (30°C) and non-permissive (37°C) temperatures in *Caulobacter* cells with a temperature-sensitive allele of *ctrA*, *ctrA401*. Aliquots were removed at the indicated times and assayed for β-galactosidase activity.

The inhibition of DNA replication prevents the accumulation of CtrA in pre-divisional cells

Our results indicate that DNA replication is required for the transcription of *ftsQA*. The lack of *ftsQA* transcription when DNA replication is inhibited suggests that either an activator of *ftsQA* transcription is missing or a repressor is present. Since CtrA is an activator of *ftsQA* transcription, we hypothesized that CtrA could be mediating the DNA replication checkpoint as part of its role as a global cell cycle regulator. CtrA is also a repressor of *ftsZ* transcription; therefore, if CtrA is inactivated when DNA replication is inhibited, *ftsZ* transcription should increase during inhibition of DNA replication. To test this, we measured the effect of inhibiting DNA replication on P_Z transcription by adding hydroxyurea to a culture of a strain containing the *ftsZ–lacZ* fusion plasmid plac290/HB2.0BP.

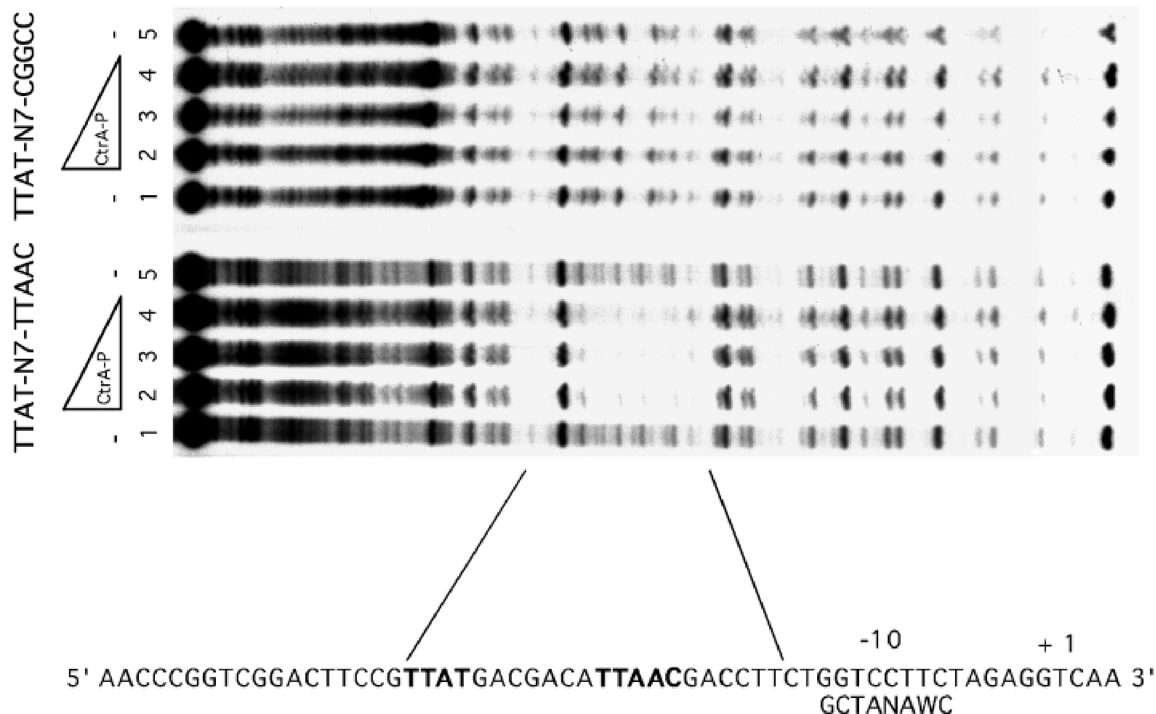

Fig. 6. DNase I footprinting of the *ftsQA* promoter by His_6-CtrA-P. A 198 bp fragment, including the CtrA-binding site TTAT-N7-TTAAC of the P_{QA} promoter, was labeled at the 3′ end by a ^{32}P-labeled oligonucleotide. The size of the resulting fragments was determined by a ^{32}P-end-labeled 10 bp ladder (Gibco). Lanes 1 and 5 contain no added CtrA-P. Lanes 2, 3 and 4 contain 126, 100 and 50 µg/ml of His_6-CtrA-P, respectively. The bottom footprint is with a wild-type template. The top footprint is with a CtrA-binding site mutant template (TTAT-N7-TTAAC to TTAT-N7-CGGCC).

The transcription from P_Z increased to ~200% of the initial level of transcription within 75 min of DNA replication inhibition (Figure 2C). This strongly suggests that inhibition of DNA replication somehow inactivates CtrA.

In order to determine if CtrA was absent when DNA replication was blocked, we tested whether the synthesis of CtrA was affected by inhibition of DNA polymerase. Strain YB1804 harbors a temperature-sensitive mutation in the DNA replication α subunit gene, *dnaE*. YB1804 was grown at the permissive temperature and was synchronized by density centrifugation. The swarmer cell fraction was divided into two aliquots; one was grown at the permissive temperature of 28°C for DNA replication and the other at the non-permissive temperature of 37°C. At the permissive temperature, cells proceeded normally through the cell cycle and were able to divide (Figure 7C, 28°C), whereas they were unable to complete cell division at the non-permissive temperature (Figure 7C, 37°C). At various times, aliquots were taken for immunoblot analysis, pulse-labeled with [8-^{3}H]dGTP to measure DNA synthesis or pulse-labeled with [^{35}S]methionine and processed for immunoprecipitation of ^{35}S-labeled CtrA. DNA synthesis analysis revealed that DNA replication took place at the permissive temperature, but not at the non-permissive temperature (data not shown). Immunoblot analysis was used to determine the concentration of CtrA during the cell cycle. At the permissive temperature, CtrA was present in swarmer cells, degraded during swarmer cell differentiation and reappeared 120 min into the cell cycle as previously described (Domian *et al.*, 1997) (Figure 7A). When DNA replication was inhibited, CtrA was present in swarmer cells, was degraded during swarmer cell differentiation, but only accumulated to a low level later in the cell cycle (Figure 7B). To ensure that the inhibition of CtrA accumulation was not due simply to heat shock, wild-type strain NA1000 was synchronized and allowed to proceed through the cell cycle at 37°C. Immunoblot analysis revealed that CtrA was present in pre-divisional cells (data not shown). Addition of hydroxyurea to a synchronized culture also prevented CtrA accumulation (M.Martin and Y.V.Brun, unpublished results).

We used an anti-CtrA antibody to immunoprecipitate pulse-labeled protein samples to determine the rate of CtrA synthesis during the cell cycle (Figure 7). At the permissive temperature for DNA replication, CtrA synthesis was undetectable in swarmer cells and peaked towards the middle of the cell cycle, consistent with previous studies of *ctrA* transcription (Quon *et al.*, 1996). At the non-permissive temperature, CtrA synthesis was undetectable in swarmer cells and increased to a very low level for the remainder of the cell cycle. This result indicates that either the rate of synthesis of CtrA was reduced substantially by the inhibition of DNA replication or that its rate of degradation was increased substantially.

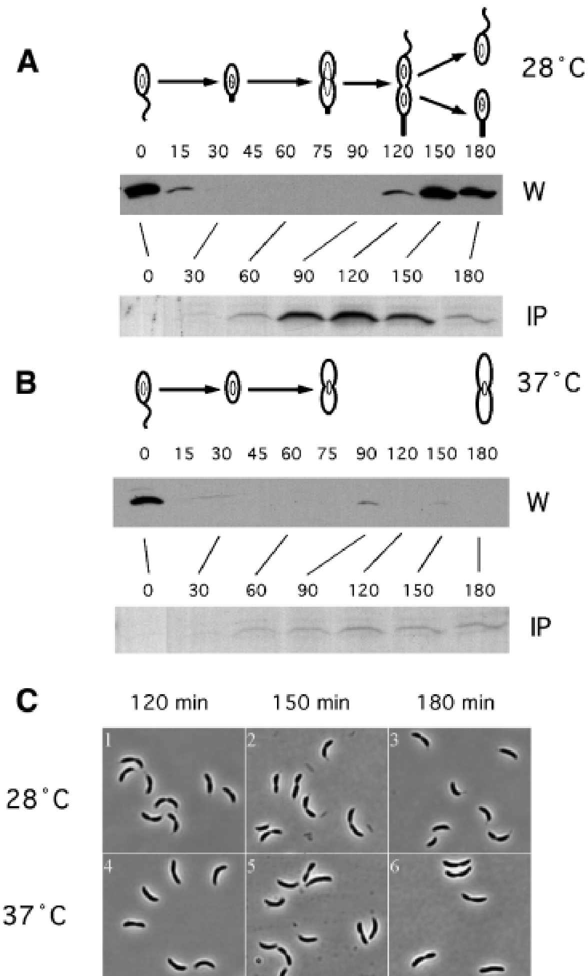

Fig. 7. The inhibition of DNA replication prevents the accumulation of CtrA in pre-divisional cells. Swarmer cells of strain YB 1804, which contains a temperature-sensitive *dnaE* allele, were collected by density centrifugation and resuspended to 0.4 OD_{600} in M2-glucose. Cells were grown for 10 min at 28°C, and the culture was split: (**A**) one culture was grown at 28°C (permissive temperature) and (**B**) one at 37°C (non-permissive temperature) and allowed to proceed through the cell cycle. Cell division occurred at 175 min at permissive temperature. Aliquots were taken at the times indicated and were immunoblotted with anti-CtrA antibody (shown above) or labeled with [^{35}S]methionine and immunoprecipitated with anti-CtrA antibody (shown below). The progression through the cell cycle as determined by microscopic examination is depicted above the immunoblots. (**C**) Panels 1–3 show the synchronized population at permissive temperature at 120, 150 and 180 min, respectively. Panels 4–6 show the synchronized population at non-permissive temperature at 120, 150 and 180 min, respectively. Note that all the cells are arrested at the pre-divisional stage. W, western blot; IP, immunoprecipitate.

Transcription of the ctrA P2 promoter is inhibited by replication inhibition

ctrA is transcribed by two promoters (Domian *et al.*, 1999). The weak promoter P1 is transcribed early and is inhibited by CtrA-P. The degradation of CtrA-P during swarmer cell differentiation allows the transcription of *ctrA* to produce a low level of CtrA which becomes phosphorylated in stalked cells (Domian *et al.*, 1997). CtrA-P then activates the strong promoter P2 in pre-divisional cells, resulting in a substantial accumulation of CtrA-P (Figure 8A). It seemed likely that the inhibition of DNA replication could prevent the activation of the P2 promoter required for the accumulation of CtrA. To test this model, we measured the cell cycle transcription from the P2 promoter using a *ctrA* P2–*lacZ* fusion in plasmid p*ctrA*-P2 (Domian *et al.*, 1999). The *dnaC* temperature-sensitive strain PC2179 containing p*ctrA*-P2 was synchronized. The swarmer cell fraction was divided into two aliquots; one was grown at the permissive

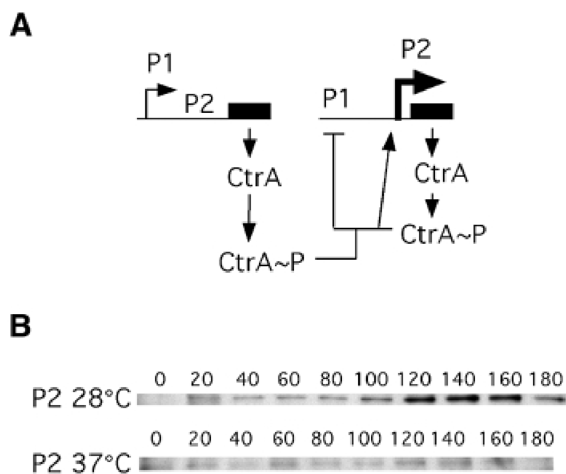

Fig. 8. The *ctrA* P2 promoter is sensitive to the inhibition of DNA replication. (**A**) Model for the regulation of *ctrA* transcription as described in the text. (**B**) Effect of DNA replication inhibition on transcription from the *ctrA* promoter, P2. Swarmer cells of strain PC2179, which contains the temperature-sensitive *dnaC303* allele, were collected by density centrifugation and resuspended to 0.2 OD_{600} in M2-glucose. Cells were grown for 10 min at 28°C and the culture was split: one culture was grown at 28°C (permissive temperature) and one at 37°C (non-permissive temperature) and allowed to proceed through the cell cycle. Cell division occurred at 180 min at permissive temperature. Aliquots were taken at the times indicated and were labeled with [^{35}S]methionine and immunoprecipitated with anti-β-galactosidase antibody. The autoradiograms show the immunoprecipitated labeled β-galactosidase. DNA synthesis was analyzed by pulsing an aliquot with [8-^{3}H]dGTP and measuring the amount of labeled DNA. DNA replication was inhibited at non-permissive temperature (data not shown).

temperature of 28°C for DNA replication and the other at the non-permissive temperature of 37°C. DNA synthesis analysis revealed that DNA replication took place at the permissive temperature, but not at the non-permissive temperature (data not shown). At the permissive temperature, transcription of the *ctrA* P2 promoter was low in swarmer and stalked cells, began to increase in early pre-divisional cells, and peaked in late pre-divisional cells as described previously (Figure 8B) (Domian *et al*., 1999). At the non-permissive temperature, transcription from the *ctrA* P2 promoter remained at a low level throughout the cell cycle (Figure 8B). Thus, transcription from the *ctrA* P2 promoter is prevented in the absence of DNA replication.

Discussion

During the *Caulobacter* cell cycle, the cell division genes *ftsZ*, *ftsQ*, and *ftsA* are expressed sequentially in an order that mimics their order of action. The transcription of *ftsZ* coincides with the replication of the chromosome (Kelly *et al*., 1998), whereas the transcription rate of *ftsQA* is maximal after the completion of DNA replication (Sackett *et al*., 1998). In this study, we show that, in addition to being a repressor of *ftsZ* transcription (Kelly *et al*., 1998), CtrA is an activator of *ftsQA* transcription and mediates the ordered transcription of *ftsZ* and *ftsQA*. Furthermore, we show that the transcription of *ftsQA* requires DNA replication and we provide evidence that this checkpoint is mediated by CtrA.

We propose that CtrA is an activator of P_{QA} transcription based on the following evidence. (i) The P_{QA} promoter, defined by deletion analysis, has an architecture similar to that of other promoters that are activated by CtrA. The –35 sequence of the P_{QA} promoter contains a putative CtrA-binding site which has only one deviation from the CtrA consensus; DNase I footprinting analysis shows that CtrA binds to this site. (ii) Transcription of P_{QA} is low in stalked cells where CtrA is absent and massively increases in pre-divisional cells when CtrA reappears. (iii) *In vivo* transcription from P_{QA} decreases when a temperature-sensitive allele of *ctrA* is inactivated. Under the same conditions, transcription of a control promoter, which does not contain a CtrA-binding site, remains unaffected. (iv) A mutation of the CtrA-binding site abolishes P_{QA} transcription and CtrA footprinting.

The ordered transcription of *ftsZ* and *ftsQA* in stalked cells can be explained fully by the opposite action of CtrA on the two promoters in question. CtrA is degraded during swarmer to stalked cell differentiation, relieving the repression of *ftsZ* transcription and DNA replication initiation. When CtrA reappears in pre-divisional cells at the end of the DNA replication period, CtrA represses *ftsZ* transcription and activates *ftsQA* transcription. Thus, the expression of the early acting *ftsZ* gene is turned on earlier than the transcription of the late acting *ftsQA* genes. The ordered transcription of *ftsZ* and *ftsQA* parallels the ordered transcription of the two *ctrA* promoters: the weak P1 promoter and the strong P2 promoter (Domian *et al*., 1999). P1 is repressed by CtrA and is transcribed at the same time as other CtrA-repressed promoters, P_Z and the origin promoter P_S. P2, whose transcription pattern mimics that of P_{QA}, is activated by CtrA. When CtrA is degraded during swarmer cell differentiation, repression of the P1 promoter is relieved, allowing P1 transcription. Later in the cell cycle, CtrA proteolysis abates and CtrA begins to accumulate and is phoshorylated in early pre-divisional cells. This results in a feedback loop that activates P2 and represses P1. Thus, CtrA acts as a molecular clock to order the expression of promoters during the cell cycle (Domian *et al*., 1999).

One feature of all identified CtrA-dependent promoters that remains to be explained is that they are not transcribed in swarmer cells, which contain a relatively high concentration of CtrA-P (Domian *et al*., 1997). A possible explanation for this observation is that a repressor is present in swarmer cells to prevent transcription of some CtrA-activated genes. In the case of *ftsQA*, removing sequences +7 to +52 from P_{QA} stimulates *ftsQA* transcription, suggesting that a repressor may bind to this region to repress transcription in swarmer cells. This is similar to a proposed role for sequences in the upstream region of the *E.coli ftsA* promoter (Dewar and Donachie, 1990). In the case of *ccrM*, methylation of the promoter is important in controlling transcription in swarmer cells. When the +11 and +16 residues of the *ccrM* promoter were mutagenized to eliminate methylation sites, transcription in swarmer cells increased from ~1 to ~78% of the activity seen in stalked cells (Stephens *et al*., 1995b). However, there are no CcrM methylation sites in the P_{QA} promoter.

Previous studies have shown that cell division in *Caulobacter* requires DNA replication (Degnen and Newton, 1972a,b; Osley and Newton, 1977, 1980; Ohta

et al., 1990). Upon inhibition of DNA replication in temperature-sensitive replication mutants and in wild-type NA1000 after addition of hydroxyurea, *Caulobacter* was able to elongate but was unable to complete cell division. P_{QA} transcription is increased substantially at the end of the DNA replication period, suggesting that P_{QA} could be the target of a DNA replication checkpoint. Indeed, DNA replication inhibition had a strong inhibitory effect on transcription from P_{QA}. This DNA replication checkpoint may be executed by inhibiting the transcription from P_{QA} in order to stop completion of cell division. P_{QA} transcription was still sensitive to the replication state in a *recA*$^-$ mutant, indicating that the inhibition of *ftsQA* transcription is not mediated by the SOS response in *Caulobacter*.

In addition, the transcription of *ftsZ*, which occurs during DNA replication, increased to ~200% of its initial level upon inhibition of DNA replication. Thus, the inhibition of DNA replication leads to opposite effects on two cell division promoters: an increase in the transcription rate of P_Z and a decrease in the transcription rate of P_{QA}, as expected for CtrA-regulated promoters. Under normal growth conditions, CtrA accumulates to high levels in pre-divisional cells. This accumulation of CtrA did not occur when DNA replication was inhibited. The absence of CtrA in pre-divisional cells when DNA replication is inhibited leads to an increase in *ftsZ* transcription and a decrease in *ftsQA* transcription. What prevents CtrA accumulation when DNA replication is inhibited? We have shown that when DNA replication is inhibited, the rate of synthesis of CtrA is low (Figure 7). Furthermore, we have shown that transcription of *ctrA* from the strong, late P2 promoter is prevented in the absence of DNA replication (Figure 8). Therefore, the regulation of the P2 *ctrA* promoter appears to be central in the DNA replication checkpoint. Under normal conditions, the weak P1 promoter activates *ctrA* transcription when CtrA-P is degraded in stalked cells. Phosphorylation of CtrA synthesized from P1 transcripts activates the strong P2 promoter, resulting in the high rate of CtrA in the pre-divisional cell. Thus, the DNA replication checkpoint could function by preventing the phosphorylation of CtrA-P or by activating its dephosphorylation.

Our results also indicate that CtrA links flagellum synthesis and DNA methylation to DNA replication since *ccrM* and *fliQ* are regulated in a manner similar to *ftsQA* (Dingwall *et al*., 1992; Stephens and Shapiro, 1993; Stephens *et al*., 1995b; Quon *et al*., 1996; Reisenauer *et al*., 1999). Other laboratories have shown that transcription of *ccrM* and *fliQ* decreases rapidly when DNA replication is inhibited (Dingwall *et al*., 1992; Stephens *et al*., 1995b) and that CtrA activates the transcription of both *ccrM* and *fliQ* (Stephens *et al*., 1995b; Quon *et al*., 1996). This work describes the absence of CtrA under conditions of DNA replication inhibition; thus, the absence of CtrA in pre-divisional cells prevents the transcription of *fliQR* and *ccrM*. In addition, the transcriptional activity of the CtrA-dependent *fliLM* operon was shown to depend on DNA replication (Stephens and Shapiro, 1993). Inhibition of DNA replication early in the cell cycle inhibited *fliLM* transcription, but inhibition of DNA replication later in the cell cycle had less effect on *fliLM* transcription. In fact, the DNA replication checkpoint was only effective until the pre-divisional cell stage when the CtrA-P concentration becomes substantial.

The mediation of a DNA replication checkpoint by a global response regulator has also been found in *B.subtilis*. The Spo0A response regulator is responsible for initiation of sporulation by acting as both an activator and a repressor of transcription (Errington, 1996). Inhibition of DNA replication at the beginning of spore development prevents sporulation (Ireton and Grossman, 1992). It was found that constitutively active Spo0A, which bypasses the need for the phosphorelay pathway, activates sporulation even in the absence of DNA replication. This shows that phosphorylation of the response regulator Spo0A mediates the DNA replication checkpoint for sporulation. *Escherichia coli* may also have non-SOS-mediated DNA replication checkpoints for cell division. It is thought that expression of the cell division gene *ftsA* requires the replication of the chromosome terminus (Tormo *et al*., 1985a,b; Grossman *et al*., 1989; Masters *et al*., 1989). This model is based on the fact that *E.coli* cells with a temperature-sensitive mutation in *ftsA* are unable to begin dividing following a shift to permissive temperature if the chromosome terminus is not replicated (Tormo *et al*., 1985a,b; Grossman *et al*., 1989; Masters *et al*., 1989). Thus, it has been postulated that transcription of the terminus either directly or indirectly induces transcription of the *ftsA* gene (Tormo *et al*., 1985a,b; Masters *et al*., 1989).

Interestingly, the chromosome partitioning genes *parA* and *parB* are essential in *Caulobacter*, and their over-expression causes defects in cell division (Mohl and Gober, 1997). This suggests the possibility that ParA and ParB are involved in a checkpoint coordinating chromosome movement and cell division. Recent experiments indicate that depletion of ParA and ParB inhibits cell division but does not inhibit transcription of *ftsQA*, suggesting that chromosome segregation defects do not prevent cell division by inhibiting CtrA synthesis (D.Mohl and J.Gober, personnal communication). Thus, there are at least two different checkpoints that couple cell division to chromosome status. The effect of replication inhibition on CtrA synthesis reported herein is similar to the effect of an *smc* mutation (Jensen and Shapiro, 1999). SMC proteins are involved in chromosome maintenance and structure in bacteria, archaea and eukaryotes. Disruption of the *Caulobacter smc* gene resulted in a temperature-sensitive cell cycle arrest that was characterized by an absence of CtrA accumulation in pre-divisional cells. However, DNA replication was not inhibited in the *smc* mutant, suggesting that some defects in chromosome organization can also prevent CtrA accumulation. The challenge will be to determine how the state of chromosome replication and dynamics are sensed and how this information is transduced to the cell division machinery.

Materials and methods

Bacterial strains, plasmids and growth conditions

Escherichia coli DH5α (Liss, 1987) was used as a host for cloning, and S17-1 (Simon *et al*., 1983) was used for conjugal transfer of plasmids to *Caulobacter* (Ely, 1991). The *E.coli* strains were grown at 37°C in LB medium supplemented with ampicillin (100 μg/ml), and tetracycline (12 μg/ml) as necessary. *Caulobacter* strains were all derivatives of strain NA1000 (Evinger and Agabian, 1977) and were grown at 30°C in PYE

medium (Poindexter, 1964) or in M2-glucose (Johnson and Ely, 1977) supplemented with nalidixic acid (20 μg/ml) and tetracycline (2 μg/ml in solid media, 1 μg/ml in liquid media) as necessary. *Caulobacter* strains used in this study include: YB1804, *dnaE* temperature-sensitive mutant (T.Lo, T.Werner, N.Din, E.M.Quardokus and Y.V.Brun, unpublished); PC2179, *dnaC303* temperature-sensitive mutant (Ohta *et al*., 1990); *ctrA401*, *ctrA* temperature-sensitive mutant (Quon *et al*., 1996); and strain CM5256, containing the *recA* mutant allele, *rec-526* (O'Neill *et al*., 1985). Strain YB682 was constructed by introducing plasmid p*ctrA*-P2 (Domian *et al*., 1999) into PC2179.

Plasmids pH10, pMSP8LC, plac290/AJK2 (Sackett *et al*., 1998), plac290/HB2.0BP (Kelly *et al*., 1998), pHB2.0 (Quardokus *et al*., 1996), pSKII+ (Stratagene) and pRKlac290 (Gober and Shapiro, 1992) were described previously. Plasmid pMS47SK was constucted by excising a *Pst*I–*Bam*HI fragment upstream of pHB2.5 in pH10 and ligating into pSKII+.

Plasmids pPQA-421LC, pPQA-123LC, pPQA-88LC and pPQA+11LC are 5′ deletions of the P_{QA} promoter region transcriptionally fused to a promoterless *lacZ* gene in pRKlac290. Each of these fragments was PCR amplified from pH10 (Sackett *et al*., 1998) with the same 3′ primer, whereas the 5′ primers differed. 5′ Primers introduced an *Eco*RI site and the 3′ primer introduced a *Hin*dIII site. Following amplification, the PCR products were digested with *Eco*RI and *Hin*dIII and ligated into pBluescript II+ (Stratagene) to form plasmids pPQA-421SK, pPQA-123SK, pPQA-88SK and pPQA+11LC. Plasmids pPQA-421LC, pPQA-123LC, pPQA-88LC and pPQA+11LC were formed by excising the fragment with *Eco*RI and *Hin*dIII and ligating them into *Eco*RI–*Hin*dIII-digested pRKlac290 which contains a promoterless *lacZ* gene (Gober and Shapiro, 1992).

Plasmid pPQAMut-421LC is equivalent to pPQA-421LC except that the CtrA-binding site was mutagenized from TTAT-N7-TTAAC to TTAT-N7-CGGCC. Mutagenesis of the CtrA-binding site was performed using the QuikChange Site-Directed Mutagenesis kit (Stratagene) on pPQA-421SK with primers PQACtrA-1 (CTT CCG TTA TGA CGA CAC GGC CGA CCT TCT GGT CCT TC) and PQACtrA-2 (GAA GGA CCA GAA GGT CGG CCG TGT CGT CAT AAC GGA AG) which introduced an *Eag*I site. Following confirmation of the mutagenesis by sequencing, the fragment was excised with *Eco*RI and *Hin*dIII and ligated into *Eco*RI–*Hin*dIII-digested pRKlac290 to form pPQAMut-421LC.

Plasmids p3′QA-24, p3′QA+7 and p3′QA+52 are 3′ deletions of the P_{QA} promoter region transcriptionally fused to a promoterless *lacZ* gene in pRKlac290. Each of these fragments was PCR amplified from pMS47SK with the same 5′ primer, whereas the 3′ primers differed. The 5′ primer introduced an *Eco*RI site and the 3′ primers introduced a *Hin*dIII site. Following amplification, the PCR products were digested with *Eco*RI and *Hin*dIII and ligated into pRKlac290, which contains a promoterless *lacZ* gene.

β-galactosidase assays of lacZ transcriptional fusions

Caulobacter NA1000 strains containing *lacZ* transcriptional fusion plasmids were assayed for transcriptional activity in mixed cultures as described previously (Miller, 1972), with the modification that cells were permeabilized with chloroform.

DNase I footprinting

DNase I footprinting of the P_{QA} promoter region was accomplished by designing primers 5′footQNew (5′ CTC CCG GCC CCA ATC CCC 3′) and 3′footQNew (5′ CGT GGT CGG CCT GCT CGG 3′) to flank the putative CtrA-binding site. The 3′ primer was phosphorylated using [γ-^{32}P]ATP and polynuclease kinase. The labeled 3′ primer and unlabeled 5′ primer were used to PCR amplify a region of 198 bp containing the CtrA-binding site of P_{QA}. The PCR product was loaded onto a 12% polyacrylamide non-denaturing gel and electrophoresed in 1× TBE. The 198 bp fragment was cut from the gel and electroeluted in 0.2× TBE at 300 V for 3 h (Davis *et al*., 1986).

His_6-CtrA was purified from strain BL21 (Novagen) containing plasmid pTRC7.4 (Quon *et al*., 1996) from the insoluble fraction of cell lysate as described in the pET system manual (Novagen) and phosphorylated with MBP-EnvZ (a gift from M.Igo; Huang *et al*., 1996). The phosphorylated His_6-CtrA was used immediately in DNase I footprinting experiments as described (Kelly *et al*., 1998).

Mixed culture transcription and DNA replication assays

To analyze the effect of DNA replication on the transcription of the promoters from the *ftsQAZ* region, a mixed population of cells containing *lacZ* transcriptional fusions (pMSP8LC, plac290/AJK2 or plac290/HB2.0BP) grown in M2-glucose medium was divided into two cultures at mid-log phase. One culture was treated with 3 mg/ml hydroxyurea (Fluka) whereas the second culture remained untreated. At 15 min intervals, 1 ml aliquots of cells were removed and pulse labeled with 15 μCi of *Trans* [^{35}S]LABEL (ICN Radiochemicals) for 5 min at 30°C or with 1 μCi of [8-^{3}H]dGTP (ICN Radiochemicals) for 2 min at 30°C. The [^{35}S]methionine-labeled samples were treated as described below for synchrony samples. The [8-^{3}H]dGTP replication assay was performed as described (Marczynski *et al*., 1990; Marczynski and Shapiro, 1992).

Cell cycle transcription assays

For synchronization, swarmer cells from a late log phase cultures were isolated by Ludox (DuPont) density centrifugation (Evinger and Agabian, 1977), washed, resuspended in M2-glucose medium and incubated with shaking at 28°C. Swarmer cells were allowed to grow for 10 min before samples were taken. At various times, 1 ml aliquots were taken: for immunoblot analysis, pulse labeled with 15 μCi of *Trans* [^{35}S]LABEL (ICN Radiochemicals) for 5 min or with 1 μCi of [8-^{3}H]dGTP (ICN Radiochemicals) for 2 min. Samples for immunoblot analysis were resuspended in 50 μl of 10 mM Tris–HCl pH 8, and 50 μl of 2× SDS loading buffer was added. A 10 μl aliquot of each sample was loaded on a 10% SDS–polyacrylamide gel and electrophoresed. Immunoblotting on these samples was performed as described below. ^{35}S-labeled cells were resuspended and lysed with immunoprecipitation wash buffer (50 mM Tris–HCl, pH 8.3, 450 mM NaCl, 0.5% Triton X-100, 4 mg/ml lysozyme). A 10 μl aliquot of each sample was precipitated with 10% trichloroacetic acid (TCA) and counted in a scintillation counter cocktail after binding to glass fiber filters GF/C (Whatman). Equal counts were immunoprecipitated with an anti-CtrA antibody (gift of Lucy Shapiro) at a 1:200 dilution or an anti-β-galactosidase antibody (Rockland) at a 1:200 dilution. The samples were electrophoresed on a 10% SDS–polyacrylamide gel, fixed, amplified with Amplify (Amersham), and the dried gel was exposed to film or to a phosphorimaging cassette.

Immunoblot analysis

Immunoblot analysis with the anti-RecA antibody (gift of Michael Cox) and the anti-CtrA antibody was performed on NA1000 cells. Equal amounts of total protein were loaded in each lane of a 12 or 15% SDS–polyacrylamide gel, respectively, and transferred to nitrocellulose (Schleicher & Schuell). Both blots were probed with a 1:10 000 dilution of primary antibody and a 1:20 000 dilution of horseradish peroxidase-conjugated anti-rabbit IgG (Gibco-BRL) pre-absorbed with acetone-powdered NA1000 (Maddock and Shapiro, 1993). The bands corresponding to RecA were quantitated by ImageQuant (Molecular Dynamics) following densitometric scanning.

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References

Bender,R.A. (1984) Ultraviolet mutagenesis and inducible DNA repair in *Caulobacter crescentus*. *Mol. Gen. Genet.*, **197**, 399–402.

Bi,E. and Lutkenhaus,J. (1993) Cell division inhibitors SulA and MinCD prevent formation of the FtsZ ring. *J. Bacteriol.*, **175**, 1118–1125.

Brun,Y., Marczynski,G. and Shapiro,L. (1994) The expression of asymmetry during cell differentiation. *Annu. Rev. Biochem.*, **63**, 419–450.

Davis,L.G., Dibner,M.D. and Battey,J.F. (1986) *Electroelution. Basic Methods in Molecular Biology*. Elsevier Science Publishing Co., Inc., New York, pp. 112–114.

Degnen,S.T. and Newton,A. (1972a) Chromosome replication during development in *Caulobacter crescentus*. *J. Mol. Biol.*, **64**, 671–680.

Degnen,S.T. and Newton,A. (1972b) Dependence of cell division on the completion of chromosome replication in *Caulobacter crescentus*. *J. Bacteriol.*, **110**, 852–856.

Dewar,S.J. and Donachie,W.D. (1990) Regulation of expression of the *ftsA* cell division gene by sequences in upstream genes. *J. Bacteriol.*, **172**, 6611–6614.

Dingwall,A., Zhuang,W.Y., Quon,K. and Shapiro,L. (1992) Expression of an early gene in the flagellar regulatory hierarchy is sensitive to an interruption in DNA replication. *J. Bacteriol.*, **174**, 1760–1768.

Domian,I.J., Quon,K.C. and Shapiro,L. (1997) Cell type-specific phosphorylation and proteolysis of a transcriptional regulator controls the G_1-to-S transition in a bacterial cell cycle. *Cell*, **90**, 415–424.

Domian,I.J., Reisenauer,A. and Shapiro,L. (1999) Feedback control of a master bacterial cell-cycle regulator. *Proc. Natl Acad. Sci. USA*, **96**, 6648–6653.

Ely,B. (1991) Genetics of *Caulobacter crescentus. Methods Enzymol.*, **204**, 372–384.

Errington,J. (1996) Determination of cell fate in *Bacillus subtilis*. *Trends Genet.*, **12**, 31–34.

Evinger,M. and Agabian,N. (1977) Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. *J. Bacteriol.*, **132**, 294–301.

Gober,J.W. and Shapiro,L. (1992) A developmentally regulated *Caulobacter* flagellar promoter is activated by 3′ enhancer and IHF binding elements. *Mol. Biol. Cell*, **3**, 913–926.

Grossman,N., Rosner,E. and Ron,E.Z. (1989) Termination of DNA replication is required for cell division in *Escherichia coli*. *J. Bacteriol*., **171**, 74–79.

Huang,J., Cao,C. and Lutkenhaus,J. (1996) Interaction between FtsZ and inhibitors of cell division. *J. Bacteriol.*, **178**, 5080–5085.

Ireton,K. and Grossman,A.D. (1992) Coupling between gene expression and DNA synthesis early during development in *Bacillus subtilis*. *Proc. Natl Acad. Sci. USA*, **89**, 1–12.

Ireton,K. and Grossman,A.D. (1994) A developmental checkpoint couples the initiation of sporulation to DNA replication in *Bacillus subtilis*. *EMBO J.*, **13**, 1566–1573.

Jenal,U. and Fuchs,T. (1998) An essential protease involved in bacterial cell cycle control. *EMBO J.*, **17**, 5658–5669.

Jensen,R.B. and Shapiro,L. (1999) The *Caulobacter crescentus smc* gene is required for cell cycle progression and chromosome segregation. *Proc. Natl Acad. Sci. USA*, **96**, 10661–10666.

Johnson,R.C. and Ely,B. (1977) Isolation of spontaneously derived mutants of *Caulobacter crescentus. Genetics*, **86**, 25–32.

Kelly,A.J., Sackett,M.J., Din,N., Quardokus,E. and Brun,Y.V. (1998) Cell cycle-dependent transcriptional and proteolytic regulation of FtsZ in *Caulobacter. Genes Dev.*, **12**, 880–893.

Liss,L.R. (1987) New M13 host: DH5aF′ competent cells. *Focus*, **9**, 3, 13.

Maddock,J.R. and Shapiro,L. (1993) Polar location of the chemoreceptor complex in the *Escherichia coli* cell. *Science*, **259**, 1717–1723.

Marczynski,G.T. and Shapiro,L. (1992) Cell cycle control of a cloned chromosomal origin of replication from *Caulobacter crescentus. J. Mol. Biol.*, **226**, 959–977.

Marczynski,G.T., Dingwall,A. and Shapiro,L. (1990) Plasmid and chromosomal DNA replication and partitioning during the *Caulobacter crescentus* cell cycle. *J. Mol. Biol.*, **212**, 709–722.

Marczynski,G., Lentine,K. and Shapiro,L. (1995) A developmentally regulated chromosomal origin of replication uses essential transcription elements. *Genes Dev.*, **9**, 1543–1557.

Masters,M., Paterson,T., Popplewell,A.G., Owen-Hughes,T. and Pringle,J.H. (1989) The effect of DnaA protein levels and the rate of initiation at oriC on transcription originating in the *ftsQ* and *ftsA* genes: *in vivo* experiments. *Mol. Gen. Genet.*, **216**, 475–483.

Miller,J.H. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Mohl,D.A. and Gober,J.W. (1997) Cell cycle-dependent polar localization of chromosome partitioning proteins in *Caulobacter crescentus. Cell*, **88**, 675–684.

Ohta,N. and Newton,A. (1996) Signal transduction in the cell cycle regulation of *Caulobacter* differentiation. *Trends Microbiol.*, **4**, 326–332.

Ohta,N., Masurekar,M. and Newton,A. (1990) Cloning and cell cycle-dependent expression of DNA replication gene *dnaC* from *Caulobacter crescentus. J. Bacteriol.*, **172**, 7027–7034.

Ohta,N., Grebe,T.W. and Newton,A. (2000) Signal transduction and cell cycle checkpoints in developmental regulation of *Caulobacter*. In Brun,Y.V. and Shimkets,L.J. (eds), *Prokaryotic Development*. American Society for Microbiology, Washington, DC, pp. 341–359.

O'Neill,E.A., Hynes,R.H. and Bender,R.A. (1985) Recombination deficient mutant of *Caulobacter crescentus. Mol. Gen. Genet.*, **198**, 275–278.

Osley,M.A. and Newton,A. (1977) Mutational analysis of developmental control in *Caulobacter crescentus*. *Proc. Natl Acad. Sci. USA*, **74**, 124–128.

Osley,M.A. and Newton,A. (1980) Temporal control of the cell cycle in *Caulobacter crescentus*: roles of DNA chain elongation and completion. *J. Mol. Biol.*, **138**, 109–128.

Poindexter,J.S. (1964) Biological properties and classification of the *Caulobacter* group. *Bacteriol. Rev.*, **28**, 231–295.

Quardokus,E.M., Din,N. and Brun,Y.V. (1996) Cell cycle regulation and cell type-specific localization of the FtsZ division initiation protein in *Caulobacter. Proc. Natl Acad. Sci. USA*, **93**, 6314–6319.

Quon,K.C., Marczynski,G.T. and Shapiro,L. (1996) Cell cycle control by an essential bacterial two-component signal transduction protein. *Cell*, **84**, 83–93.

Quon,K.C., Yang,B., Domian,I.J., Shapiro,L. and Marczynski,G.T. (1998) Negative control of DNA replication by a cell cycle regulatory protein that binds at the chromosome origin. *Proc. Natl Acad. Sci. USA*, **95**, 120–125.

Reisenauer,A., Quon,K. and Shapiro,L. (1999) The CtrA response regulator mediates temporal control of gene expression during the *Caulobacter* cell cycle. *J. Bacteriol.*, **181**, 2430–2439.

Sackett,M.J., Kelly,A.J. and Brun,Y.V. (1998) Ordered expression of *ftsQA* and *ftsZ* during the *Caulobacter crescentus* cell cycle. *Mol. Microbiol.*, **28**, 421–434.

Simon,R., Prieffer,U. and Puhler,A. (1983) A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in gram-negative bacteria. *Biotechnology*, **1**, 784–790.

Stephens,C.M. and 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**, 1169–1179.

Stephens,C., Jenal,U. and Shapiro,L. (1995a) Expression of cell polarity during *Caulobacter* differentiation. *Semin. Dev. Biol.*, **6**, 3–11.

Stephens,C., Zweiger,G. and Shapiro,L. (1995b) Coordinate cell cycle control of a *Caulobacter* DNA methyltransferase and the flagellar genetic hierarchy. *J. Bacteriol.*, **177**, 1662–1669.

Stove,J.L. and Stanier,R.Y. (1962) Cellular differentiation in stalked bacteria. *Nature*, **196**, 1189–1192.

Tormo,A., Dopazo,A., de la Campa,A., Aldea,M. and Vicente,M. (1985a) Coupling between DNA replication and cell division mediated by the FtsA protein in *Escherichia coli*: a pathway independent of the SOS response, the 'TER' pathway. *J. Bacteriol.*, **164**, 950–953.

Tormo,A., Fernández-Cabrera,C. and Vicente,M. (1985b) The *ftsA* gene product: a possible connection between DNA replication and septation in *Escherichia coli. J. Gen. Microbiol.*, **131**, 239–244.

Weinert,T. (1998) DNA damage and checkpoint pathways: molecular anatomy and interactions with repair. *Cell*, **94**, 555–558.

Wu,J., Ohta,N. and Newton,A. (1998) An essential, multicomponent signal transduction pathway required for cell cycle regulation in *Caulobacter. Proc. Natl Acad. Sci. USA*, **95**, 1443–1448.

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