A developmentally regulated chromosomal origin of replication uses essential transcription elements

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Only one of the two chromosomes in the asymmetric Caulobacter predivisional cell initiates replication in the progeny cells. Transcription from a strong promoter within the origin occurs uniquely from the replication-competent chromosome at the stalked pole of the predivisional cell. This regulated promoter has an unusual sequence organization, and transcription from this promoter is essential for regulated (cell type-specific) replication. Our analysis defines a new class of bacterial origins and suggests a coupling between transcription and replication that is consistent with the phylogenetic relationship of Caulobacter to the ancestral mitochondrion.

[Key Words: Caulobacter; origin of replication; transcription; polar development; hemE]

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Caulobacter crescentus offers the opportunity to study the control of DNA replication in the context of a developmental cell cycle. The C. crescentus predivisional cell expresses a remarkable asymmetry that polarizes cellular components so that every cell division yields two distinct cell-types: a motile nonreplicative swarmer cell and a sessile replicative stalked cell. The swarmer cell later differentiates into a stalked cell to grow and divide asymmetrically (for recent reviews, see Newton and Ohta 1990; Brun et al. 1994). In addition to distinct surface morphologies, the progeny swarmer and stalked cells receive newly replicated chromosomes that have different sedimentation coefficients (Evinger and Agabian 1979, Swoboda et al. 1982, Gober et al. 1991a), differentially transcribed genes (Gober et al. 1991a, b, Wingrove et al. 1993), as well as different replicative abilities (Marczynski and Shapiro 1992).

The great diversity of bacteria implies that they have evolved sophisticated systems to coordinate DNA replication with their diverse modes of proliferation. Unfortunately, critical information is lacking for most bacterial species regarding the organization of their origins of replication and the coordination of replication with the cell cycle. The C. crescentus chromosome, as is the case for Escherichia coli and Bacillus subtilis, uses one origin of replication (Lott et al. 1987, Dingwall and Shapiro 1989; Marczynski and Shapiro 1992). When placed on a nonreplicating plasmid, the cloned C. crescentus origin (Cori) supports autonomous plasmid replication (Marczynski and Shapiro 1992). In contrast to other plasmids, whose replication occurs in both swarmer and stalked cells (Marczynski et al. 1990), "Cori-driven" plasmid replication coincides with the initiation of chromosome replication in the stalked cell (Marczynski and Shapiro 1992), indicating that the cloned Cori DNA responds to the same regulatory factors as the chromosome. This provides a powerful system in which to study the molecular switch between chromosomes that are active or inactive for replication. A cell's commitment to replicate its genome is analogous to its commitment to differentiate by transcribing specific genes, and both processes are thought to share similar regulatory principles. Studies in E. coli have provided a detailed description of a replication initiation process that requires the ordered assembly of replication proteins (DnaA, helicase, primase, DNA polymerase, and topoisomerase subunits), collectively termed the replisome (Kornberg and Baker 1992), at a unique origin sequence. Targeted replisome assembly is conceptually similar to the action of transcriptional enhancer elements that load and activate RNA polymerase and transcription factors at specific promoters (Mitchell and Tjian 1989; Kustu et al. 1991; Roeder 1991; Heintz et al. 1992). Transcriptional enhancer elements also appear to be auxiliary components of several viral origins of replication (DePamphilis 1988; Guo and DePamphilis 1992; Li and Botchan 1993) and at least one yeast chromosomal origin of replication (Marahrens and Stillman 1992).

We report the DNA sequence requirements and functional organization of the C. crescentus chromosomal origin and demonstrate that an origin–internal promoter is important for the regulation of DNA replication. Tran-
cription from this promoter anticipates chromosome replication in the progeny cells and occurs preferentially at the stalked pole of the predivisional cell. Our results imply that the differential activity of transcription factors at the two poles affects the switch between replicating and nonreplicating chromosomes.

**Results**

**Novel DNA sequence requirements of the C. crescentus origin of replication**

The cloned Cori supports autonomous plasmid replication, and Cori-directed plasmid replication coincides with the onset of chromosomal DNA synthesis (Marczynski and Shapiro 1992). These results imply that this DNA region contains cell cycle control elements that restrict chromosome replication to the stalked cells. To define the DNA sequences required for regulated replication in *C. crescentus*, we mutagenized the Cori region systematically (Fig. 1A). Randomly generated deletions with *BamHI* linker insertions (shown as boxes numbered 1–27), as well as site-directed linker insertions and base-pair changes (shown as dagger signs a–h) were created throughout the Cori region. These mutated origins were introduced into *C. crescentus* on plasmids that cannot replicate without the functional Cori sequence, and replication function was assayed by scoring for Cori-plasmid maintenance. As illustrated for six selected Cori-plasmids in Figure 1B,C, we observed three classes of Cori-plasmids, designated Rep+, Rep+/−, and Rep−. Mutated Cori-plasmids were designated Rep+ when they were indistinguishable from unmutated Cori-plasmids by these criteria: high frequency transformation (10⁶–10⁷ colonies/µg of DNA), a large colony morphology (Fig. 1B, plates 1,2), and efficient Cori-plasmid extraction from liquid cultures (Fig. 1C, lanes 1,2). Cori-plasmids designated as Rep+/- gave comparable transformation frequencies, but their colonies were tiny (Fig. 1B, plates 3,4), and their plasmid yields were low from liquid cultures (Fig. 1C, lanes 3,4). The extracted Rep+/- plasmids not visible on ethidium bromide-stained agarose gels, and although Rep+...
Cori–plasmids were maintained indefinitely by subculturing, Rep + /− Cori–plasmids soon lost their autonomous replication state and integrated into the genome [data not shown]. Cori–plasmids designated as Rep gave low frequency transformation [<10 colonies/μg of DNA indistinguishable from background, and these few colonies could be accounted for by spontaneous acquisition of antibiotic resistance.

Cori–plasmids containing randomly generated deletions/insertions [Fig. 1A, boxes 1–27] all fall into either the Rep + or the Rep − classes. These mutations define an essential origin of replication whose left boundary lies between positions +137 and +198, whose right boundary lies between positions +635 and +714, and whose minimal length is between 437 and 577 bp.

DNA sequence motifs that are common to other bacterial origins of replication as well as those that are unique to Cori are contained within the essential DNA region defined by these mutations. At the E. coli chromosomal origin of replication, the DnaA protein, bound to specific sites [the "DnaA boxes"], promotes the unwinding of an adjacent AT-rich region (Bramhill and Kornberg 1988a; Kowalski and Eddy 1989) and subsequent replisome assembly at this site (Bramhill and Kornberg 1988b). The essential Cori region contains two DnaA box homologies that are potential binding sites for the DnaA protein (Yoshikawa and Ogasawara 1991; Zweiger and Shapiro 1994). Site-directed point mutations inside one Cori DnaA box [Fig. 1A, dagger h] abolished replication [Marczynski and Shapiro 1992]. The other two potential DnaA box homologies fall within a Cori region that is not required for replication [deletions/insertions 24–27]. Also within the essential origin is an exceptionally AT-rich domain [85% AT between positions +251 and +290] that is present in other bacterial origins of replication [Bramhill and Kornberg 1988b].

The replication region also contains sequence motifs that are unique to Cori. Three perfect 9-mer motifs and two 8/9-bp 9-mer matches [spanning the AT-rich region] fall within the essential Cori region. Deletions/insertions 11, 29, and 30 [Figs. 1A and 3D; Table 1] demonstrate that the AT-rich region is essential. Targeted mutagenesis experiments demonstrated that all three perfect 9-mer motifs are essential for replication [Fig. 1A, daggers c,d,f,g; Table 2]. Adjacent to the AT-rich region is a cluster of four 8-mer motifs, and targeted mutagenesis experiments [Fig. 1A, daggers a,b; Fig. 3C, pGM1031 and pGM1032], as well as deletions/insertions 10 and 28 [Fig. 1A; Table 1] demonstrate that these motifs are essential for replication.

### Two promoters overlap the essential C. crescentus origin of replication

An open reading frame that overlaps the essential Cori sequence was identified by DNA sequence analysis as a homolog of the E. coli hemE gene [GenBank accession no. U13664]. Sequence analysis revealed a 44% amino acid identity and a 61% similarity to human uroporphyrinogen decarboxylase [UroD] [EC 4.1.1.37], an intermediate enzyme in heme biosynthesis (Romeo et al. 1986). The E. coli hemE gene encodes UroD, and we have adopted the bacterial nomenclature by convention [Bachmann 1990]. UroD protein homology extends the entire length of the C. crescentus hemE gene [beginning at position +201], and we have performed protein fusion experiments to confirm that the HemE initiator AUG codon begins at position +201 [data not shown]. The mutational analysis shown in Figure 1A indicates that the 5' portion of the hemE gene [beginning at position +201] overlaps an essential region of the C. crescentus origin suggesting that hemE transcription from inside the Cori region may influence replication.

To examine the role of hemE transcription in the control of replication, we identified the hemE promoters and measured promoter activity during the C. crescentus cell cycle. The hemE promoters were located initially by constructing transcriptional fusions between a promoterless lacZ reporter gene and subclones from the Cori region [Fig. 2]. Constructs 1–3, which retain a large portion of the region 5' to hemE, produced between 2500 and 3000 Miller units. Constructs 4–8, which form a progressive deletion series, demonstrated promoter activity directly upstream of the hemE protein coding sequence. Construct 8, which deleted all hemE 5'-noncoding sequences up to +201, lost all promoter activity. Constructs 9–12, where the lacZ gene was inserted just upstream of the hemE protein-coding sequence, produced progressively higher levels of lacZ expression as rightward deletions removed the 8-mer motifs. This suggests that expression from a strong promoter is impeded.

**Table 1.** Deletions and insertions inside the Cori region

| Mutation | End points (bp) | Mutation | End points (bp) |
|----------|----------------|----------|----------------|
| 1        | −504/B/−503    | 16       | +385/B/+420    |
| 2        | −427/B/−426    | 17       | +397/B/+390    |
| 3        | −337/B/−318    | 18       | +395/B/+454    |
| 4        | −234/B/−230    | 19       | +511/B/+577    |
| 5        | −72/B/−53      | 20       | +615/B/+911    |
| 6        | +8/B/+60       | 21       | +635/B/+975    |
| 7        | +97/B/+100     | 22       | +714/B/+814    |
| 8        | +137/B/+198    | 23       | +714/B/+751    |
| 9        | +185/B/+322    | 24       | +718/B/+836    |
| 10       | +202/B/+212    | 25       | +764/B/+816    |
| 11       | +270/B/+300    | 26       | +767/B/+812    |
| 12       | +308/B/+330    | 27       | +813/B/+885    |
| 13       | +297/B/+332    | 28       | +212/H/+244    |
| 14       | +314/B/+345    | 29       | +254/+ +287    |
| 15       | +326/B/+353    | 30       | +259/+ +274    |

*Numbers indicate the wild-type Cori base pair positions [Marczynski and Shapiro 1992] present on the left and right sides of each deletion/insertion shown in Figs. 1A and 3C-D. (/B/) An intervening BamHI linker, GGCGGATCCCC, (/H/) an intervening HindIII linker, AAGCTTGC, (/−/) no intervening DNA. Mutations 1–30 correspond to the Cori plasmid series pGM1001–pGM1030 in Table 3.*
Table 2. Directed mutations inside the Cori region

| Mutation | Target | Replication | Description |
|----------|--------|-------------|-------------|
| a        | 8-mer  | +/-         | [see Fig. 3C, pGM1031] |
| b        | 8-mer  | +/-         | [see Fig. 3C, pGM1032] |
| c        | 9-mer  | -           | [GGGTACCC] insert at +395 [HpaI] |
| d        | 9-mer  | -           | [AATT] insert at +447 [EcoRI] |
| e        | SmaI site | +            | [GGGTACCC] insert at +627 [SmaI] |
| f        | 9-mer  | +/-         | GTTAA......TTAA base pair changes at +654–657 to CGCTA......TTAA |
| g        | 9-mer  | -           | [GGGTACCC] insert at +667 [HpaI] |
| h        | DNA A box | -             | TGATCCACA base pair changes at +677 and +678 to TGAGACACA |

Mutations a–h correspond to dagger symbols in Fig. 1A and plasmid series pGM1031–pGM1038 in Table 3.

by the 8-mer cluster. Construct 12, with lacZ inserted at the BgIII site, produced >11,000 Miller units, but construct 13, with only an extra 10-bp rightward deletion, produced only 2100 Miller units. Further rightward deletions past the AT-rich region reduced lacZ expression close to background levels (constructs 14–16). These results indicate that a strong promoter lies directly on the right side of the BgIII site inside the AT-rich region, and comparison of lacZ expression from constructs 7 and 8 demonstrates a second weaker promoter on the left side of this BgIII site (between positions +201 and +254), because this region by itself produced ~2000 Miller units.

These observations are summarized by the model drawn at the bottom of Figure 2. A weak promoter contributes ~2000 Miller units and an upstream strong promoter contributes ~10,000 Miller units, of which all but ~1000 are impeded by downstream sequences overlapping the 8-mer cluster. A mechanism for impeding strong promoter expression is suggested by potential secondary structure in the RNA leader sequences, as described below (Fig. 8A).

Primer extension assays were used to confirm this two-promoter model. A primer internal to the hemE protein-coding sequence revealed transcripts initiating at positions +201 and +251 (Fig. 3A). A primer at the start of the hemE protein-coding sequence revealed a single transcription start site at position +251/252 (Fig. 3B). These transcriptional start sites agree with the lacZ reporter experiments shown in Figure 2 and additional lacZ reporter experiments described below in Figure 3C,D.

The DNA sequences directly upstream of each hemE RNA start site are homologous to established promoters (Fig. 3C,D). Overlapping the 8-mer cluster is a consensus sequence derived from a number of $\sigma^{70}$-type C. crescentus promoters (B. Ely, pers.). This weak hemE promoter has correct spacing between the +1, −10, and −35 regions, and it matches the proposed −10 and −35 consensus in 8/9 positions (Fig. 3C). Its relative transcript-
Figure 3. Sequences required for transcription and replication within the origin of replication. (A,B) Primer extension analysis of both hemE promoters using \( {^32} \)P-labeled oligonucleotide primers complementary to an internal portion of the HemE-coding sequence (5' position +120 through +137) (A) and the amino terminus of HemE (5' position +181 through +203) (B). Lanes 1–4 (A) and 1–5 (B) present a series of primer extensions carried out at progressively increasing temperature using wild-type C. crescentus (NA1000) chromosomal RNA, as described in Materials and methods. Dideoxy-sequencing ladders served as the size standards. Numbered arrows mark the RNA start sites aligned with the Cori DNA base pair positions shown in C and D. (C,D) Sequences of two hemE promoters and analysis of transcriptional and replicative abilities of mutations in the promoter regions. The bent arrows position the primer extension results from A and B over the Cori DNA sequence directly upstream of hemE. Boxed sequences in C show the four 8-met motifs, and the connected ovals in D show the two 9-mer motifs that are present on the wild-type plasmid pGM946. Established promoter sequences and the inferred hemE promoter -10 and -35 elements are aligned above the wild-type sequence. Site-directed base pair changes and/or deletions present on mutant Cori-plasmids are shown below. Deletions are indicated by open boxes with dots, and base changes are shown with dashes to indicate the unchanged bases. Replication was assayed by electroporating these Cori-plasmids (containing the −633 to +998 Cori region) into C. crescentus strain LS108 and testing plasmid replication, as described in Fig. 1B,C. Transcription was assayed by testing Cori-plasmid subclones in the lacZ reporter system described in Fig. 2. In C, weak promoter transcription of each mutant origin was assayed by subcloning the PstI–BglII Cori DNA (+1 to +254) into pRK290 lacZ; in D, strong promoter transcription was assayed by subcloning the AflII–EcoRI Cori DNA (+250 to +452) into pRK290 lacZ. These lacZ reporter plasmids correspond to pGM2021–pGM2028 in Table 3. The transcription assay results, presented in Miller units, are the averages of at least three separate experiments with S.D. < 10%.

AUG initiation codon) is unusual but not without precedent [Shean and Gottesman 1992]. Only the weak promoter DNA (from PstI at +1 to BglII at +254) was used in the lacZ transcriptional reporter experiments shown in Figure 3C. Although the wild-type weak promoter DNA [from pGM946] produced 1780 Miller units, a deletion of the −35 region (pGM1028) and base pair changes that overlap the −10 region without changing the spacing (pGM1010) produced only 230 and 90 Miller units, respectively (Fig. 3C). Because these mutations were predicted to impair the −35 and −10 promoter elements, respectively, clearly a functional promoter resides directly upstream of the hemE protein-coding sequence inside the 8-mer cluster.

In a similar manner, we demonstrated that a strong
promoter overlaps two 9-mer motifs inside the AT-rich region. Only the strong promoter DNA (from A/fII at +250 to EcoRI at +452) was used in the lacZ transcriptional reporter experiments shown in Figure 3D. Although the wild-type strong promoter DNA (from pGM946) produced 12,430 Miller units, the substitution of 3 G’s for 3 T’s in the conserved –10 region (pGM1303), a deletion of the –10 region (pGM1029), an extensive deletion of both –10 and –35 region (pGM1029) and a deletion and base change in the –35 region (pGM1011) all produced significantly lower Miller units (Fig. 3D). Because all four mutations are predicted to impair the –10 and/or –35 promoter elements, clearly a second promoter resides in the AT-rich region.

Impaired transcription correlates with impaired replication

All mutations that reduced hemE transcription, from either the weak promoter (Fig. 3C) or the strong promoter (Fig. 3D), also impaired replication. This suggests that transcription is required for replication. However, this analysis is complicated by the overlap between promoter and replication elements. For the weak promoter, 8/9 of the –10 and –35 promoter base pairs overlap the 8-mer elements, and this precludes a mutational separation between these DNA sequences (Fig. 3C). However, as targeted 8-mer base pair changes (in pGM1031 and pGM1032) impair replication without reducing transcription from the weak promoter (Fig. 3C), we speculate that the four 8-mer motifs and not transcription from the weak promoter are required for Cori replication.

For the strong promoter, 6/9 of the C. crescentus σ70 consensus promoter base pairs overlap the 9-mer replication elements (Fig. 3D). The directed mutation analysis in Figure 1 demonstrated that the consensus 9-mer motifs are essential for autonomous Cori plasmid replication. A comparison between the C. crescentus σ70 consensus and the R. meliloti hemA homology suggests that an important portion of the –10 promoter element lies outside the 9-mer elements, implying that a separation between replication and transcription elements is possible. Therefore, we changed the three conserved base pairs (at positions 259–261) from T’s to G’s (pGM1303). As shown in Figure 3D, comparison of the wild-type promoter in pGM946 with the –10 promoter mutation in pGM1303 revealed that replication is significantly impaired when strong promoter transcription is reduced from 12,430 to 2150 Miller units. Because the pGM1303 point mutations do not affect the 9-mer elements, we infer that transcription from the strong promoter is required for Cori replication. Apparently, transcription from the strong promoter contributes a replicative function that is separable from the replicative function contributed by the 9-mer elements.

Cell-type-specific control of transcription within the C. crescentus origin of replication

To examine further the function of the two hemE promoters in the control of replication, their transcription was assayed during the developmental cell cycle. Swarmer cells were isolated and allowed to proceed synchronously through the developmental cell cycle, and at the indicated times, cells were removed and assayed for hemE transcription (Fig. 4). As described in Materials and methods, this assay measures mRNA transcription through the lacZ reporter gene. When positioned down-stream of both hemE promoters, the lacZ gene was transcribed uniformly in all cell types (Fig. 4A). However, when the lacZ gene was driven solely by the upstream strong promoter, the lacZ reporter exhibited significant cell cycle variation (Fig. 4B). A PhosphorImager quantitation of this experiment is presented in Figure 4C. Na-
scent swarmer cells produced negligible amounts of lacZ mRNA, but as the swarmer cells developed into stalked cells, the level of lacZ mRNA increased. Peak transcription was observed at ~100 min, during the stalked cell phase. Transcription then decreased in the predivisional cells to ~50% of its peak value. When cell division occurred, at ~150 min, and DNA replication resumed in the progeny stalked cells, transcription again increased from the strong promoter (Fig. 4B,C).

Because the hemE strong promoter is virtually inactive in the nascent swarmer cells, but highly active in the nascent stalked cells, we determined the precise time and polar position before cell division that this transcriptional switch occurs. Figure 4C indicates that strong promoter transcription decreased ~50% in the predivisional cells, implying that either transcription decreased equally at both cell poles or that transcription decreased preferentially at the swarmer cell pole. To distinguish these possibilities, predivisional cells (containing the strong promoter lacZ transcriptional fusion) were pulse labeled for 5 min with [35S]methionine and chased with unlabeled methionine while they continued to grow and divide (Fig. 5A). The nascent swarmer and stalked cells were isolated, and the 116-kD β-galactosidase reporter protein was immunoprecipitated from equal amounts of 35S-labeled cell extracts (Fig. 5A). The presence of the 35S-labeled β-galactosidase in the progeny cells reports the subcellular compartment (swarmer pole vs. stalked pole) of the predivisional cell containing the chimeric mRNA at the time of the [35S]methionine pulse. It has been demonstrated previously that the β-galactosidase protein shows no inherent bias toward either pole. However, if a flagellin gene promoter directs lacZ transcription, then in this protocol the β-galactosidase protein is recovered preferentially in the progeny swarmer cells (Gober et al. 1991b). As expected, the 25- and 27-kD flagellin proteins are synthesized exclusively at the swarmer pole of these same predivisional cells (Fig. 5A). In contrast, when the strong hemE promoter drives lacZ transcription, then in this protocol the β-galactosidase synthesis occurs primarily at the stalked cell pole before cell division (Fig. 5A). This indicates that the strong promoter is transcribed primarily from the chromosome at the stalked pole before cell division.

Stalked pole-specific transcription of the strong hemE promoter might be attributable to selective activation at...
the stalked pole or selective repression at the swarmer pole. To distinguish between these two mechanisms, we modified the polar transcription protocol shown in Figure 5A, by varying the time of the [35S]methionine pulse between 0 and 40 min before cell division (Fig. 5B). The progeny swarmer and stalked cells were isolated, and the relative amount of reporter protein was quantitated by immunoprecipitation and 35S-labeled PhosphorImaging, as described in Materials and methods. Transcription at the stalked pole was found to remain constant, whereas transcription at the swarmer cell pole decreased before cell division (Fig. 5B). One interpretation of these transcription kinetics is that the hemE strong promoter is repressed selectively at the swarmer pole of the predivisional cell.

The preferential transcription of the hemE strong promoter at the stalked pole (Fig. 5A,B) is probably mediated by the two 9-mer motifs that overlap both the −10 and −35 promoter elements (see Fig. 3D). The deletion in pGM1030 removes sequences from both 9-mer motifs while retaining significant transcription activity (Fig. 3D). If the 9-mer sequences contribute to the differential transcription of the strong promoter within Cori, then the mutated 9-mer sequences in pGM1030 should result in a loss of stalked pole transcription preference. Consistent with this hypothesis, the polar transcription experiments in Figure 5, C and D, which parallel those in the preceding panels for the wild-type promoter, demonstrate that this mutant promoter has lost all transcriptional preference for the stalked pole. The mutant promoter possesses a small but consistent transcription bias for the opposite swarmer pole (Fig. 5D). Therefore, stalked pole-specific regulatory sequences overlap the AT-rich region inside Cori. Although the experiment shown in Figure 5B suggests that a swarmer pole-specific repressor binds the 9-mer sequences, we cannot rule out the possibility that transcription is activated preferentially at the stalked pole. The experiment presented in Figure 5D shows that the pGM1030 mutation both decreased basal transcription and yielded equal transcription at both poles.

To confirm that stalked pole-specific transcription is a special property of the hemE strong promoter, we repeated the polar transcription protocol for each of four lacZ transcription fusions that assayed the combined hemE promoters (Fig. 6A), the hemE weak promoter alone (Fig. 6B), the wild-type strong promoter alone (Fig. 6C), and the pGM1030 mutant strong promoter alone (Fig. 6D). The ratio of 35S-labeled protein recovered in stalked versus swarmer cells (St/Sw) as a function of the time of the pulse label before cell division is shown in each case. Most proteins, such as RNA polymerase (ββ′) subunits, partition equally to both progeny cells and show a St/Sw ratio of ~1.0, whereas flagellin proteins partition exclusively to the swarmer cells (Gober et al. 1991b) and show a St/Sw ratio of <0.1 (Fig. 6). Transcription from the combined hemE promoters, which includes the 8-mer impedance region (Fig. 6A), from the weak hemE promoter alone (Fig. 6B) and from the mutant strong promoter alone (Fig. 6D), showed no stalked pole bias. Only when lacZ transcription was driven by the hemE wild-type strong promoter alone did its mRNA accumulate preferentially at the stalked pole of the predivisional cell (Fig. 6C).

**Figure 6.** Only the wild-type strong hemE promoter exhibits stalked pole-specific transcription. (A–D) Time courses before cell division of the polar localization ratio, defined as the ratio between transcription activity arising from the stalked and swarmer cell poles (St/Sw) of the predivisional cell. Each experiment used the following lacZ transcription reporter constructs: (A) the native two-promoter system; (B) the weak promoter alone; (C) the wild-type strong promoter alone; and (D) a mutant strong promoter alone. These experiments were performed as described in Fig. 5 and used lacZ transcriptional reporter constructs 3, 7, 11 (Fig. 2), and the lacZ construct from pGM1030 (Fig. 3D) shown symbolically in A–D, respectively. Equal amounts of 35S-labeled protein extracts from progeny stalked cells and swarmer cells were analyzed for each time point. β-Galactosidase and flagellins (25- and 27-kD proteins) were assayed by immunoprecipitation, and the RNA polymerase (ββ′ subunits) were observed directly on total cytosolic protein gels. Bands were quantified by PhosphorImaging and plotted as shown.
Abolishing differential transcription from the two poles of the predivisional cell abolishes regulated replication in the progeny cells

The preceding experiments imply that stalked pole-specific transcription from the hemE strong promoter is required for stalked cell-specific replication. To test this hypothesis, cell type-specific Cori-plasmid replication was assayed using plasmid constructs with either the wild-type (stalked pole-specific, Fig. 5A,B) or the pGM1030 deletion mutant (nonspecific; Fig. 5C,D) hemE strong promoters, described above. For these experiments, we used a sensitive replication assay based on the observation that C. crescentus DNA is always fully methylated at the A nucleotide of the GANTC sequence on both DNA strands before replication [Zweiger et al. 1994]. The GANTC methyltransferase is only present in late predivisional cells, therefore, DNA replication produces two hemimethylated molecules whose hemimethylated state persists until the end of the cell cycle. When the GANTC sequence overlaps a restriction endonuclease site, for example, ClaI or HincII, endonuclease cutting is blocked when both DNA strands are methylated, and the hemimethylated state is revealed by the acquired ability to cut at these sites on one of the two newly replicated DNA molecules. As reported previously, the chromosomal HincII site upstream of the dnaA gene is fully methylated in swarmer cells, and it becomes hemimethylated when swarmer cells differentiate into stalked cells [Zweiger et al. 1994]. Because the C. crescentus dnaA gene is only ~6 kb away from Cori, we used this HincII site to assay the initiation of chromosomal replication. Cori-plasmid replication was monitored similarly by inserting an oligonucleotide-bearing overlapping ClaI and GANTC methylation sites.

Using this methylation assay, the replication kinetics of wild-type Cori-plasmids and mutant pGM1030 hemE strong promoter Cori-plasmids were compared with that of the chromosome during the swarmer to stalked cell transition period of the cell cycle. Swarmer cells containing these Cori-plasmids were prepared and analyzed as they differentiated synchronously into stalked cells. Both plasmid and chromosomal DNA were prepared, the percentage of hemimethylated molecules was determined by Southern blot analysis, and the corresponding percentage of replicated molecules was quantitated as detailed in Materials and methods.

The results presented in Figure 7 demonstrate that the Cori-plasmid with the mutant hemE strong promoter, unlike the wild-type Cori-plasmid, fails to initiate replication synchronously when swarmer cells differentiate into stalked cells. Chromosomal replication served as an internal control, and in both experiments (Fig. 7A,B), the chromosomes initiated replication with identical kinetics. However, although wild-type Cori-plasmid replication exhibited a steep sigmoidal curve that closely tracked the sigmoidal curve of the chromosome (Fig. 7A), the mutant Cori-plasmid exhibited a linear replication curve distinct from that of the chromosome and indicative of random replication. This linear accumulation of replicated plasmids implies that the mutant Cori-plasmids replicated at the same rate throughout most of the cell division cycle. In contrast, the sigmoidal curve implies that the wild-type Cori-plasmid replicated primarily during the brief 10- to 20-min period spanning the swarmer to stalked cell transition at the start of the

Figure 7. Wild-type and mutant Cori-plasmid replication was measured and compared to chromosomal replication by the methylation state assay reported previously [Zweiger et al. 1994]. Wild-type Cori-plasmid (pGM946) and mutant hemE strong promoter Cori-plasmid (pGM1030) were modified so that they contained the RK2 par sequences (Roberts et al. 1990) between the HindIII and KpnI sites, and the following oligonucleotide sequence 5'-CTCGAGTCAGGCTCATCGATTCGATCGGCGGATCC-3', containing the ClaI and methylation site overlap, between the XhoI and BamHI sites. These are designated as pGM1064 and pGM1065, respectively (Table 3). Swarmer cells bearing these plasmids were isolated and allowed to differentiate into stalked cells by release into PYE medium. At the indicated times, both plasmid and chromosomal DNA were prepared separately and analyzed by Southern blot and PhosphorImaging techniques as described in Materials and methods. Chromosomal DNA was digested with HincII, and the Southern blots were hybridized with a 420-bp piece of 32P-labeled DNA spanning the 5' region of the C. crescentus dnaA gene (Zweiger and Shapiro 1994). Plasmid DNA was linearized with PstI and then redigested with excess ClaI. Cori-plasmid Southern blots were hybridized with 32P-labeled pGM946 vector DNA between the ClaI and PstI sites. [A,B] The time course of the percentage of replicated molecules; chromosomal DNA (○,△) and plasmid DNA (■,▲) were obtained from analyses of images of the corresponding Southern blots. [C,D] Autoradiographs of the corresponding wild-type and mutant Cori-plasmid Southern blots. The chromosomal DNA southern blots are not shown.

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DNA synthesis period. These wild-type Cori–plasmid replication results agree with those reported previously using a radioactive pulse-labeling method to assay replication [Marczynski and Shapiro 1992]. Because the only difference between the wild-type and mutant Cori–plasmids is a 14-bp deletion that abolishes stalked pole-specific transcription in the predivisional cell (figs. 5C,D and 6D), we conclude that stalked pole-specific transcription from the hemE strong promoter is required to regulate replication in the progeny cells.

The stalked pole-specific hemE RNA originating from within the origin of replication is not translated

The 5′-leader sequence of the strong hemE promoter mRNA has the potential to form secondary structures that effectively bury the hemE AUG initiation codon and block ribosome binding [fig. 8A]. The majority of the base pairs in this RNA structure are formed by the four 8-mer motifs. The 5′ end of the downstream weak promoter hemE mRNA lacks this structure, and initiates transcription at the start of the AUG for the HemE coding sequence. Although this hemE mRNA lacks a Shine and Dalgarno (S.D.) sequence, it possesses a “downstream box” that is complementary to a region of the C. crescentus 16S rRNA (fig. 8A), as is the case for the λ phage cl gene (Shean and Gottesman 1992). The proximity of the hemE downstream box to the AUG and the presence in C. crescentus 16S rRNA of a domain utilized for these base pairings are directly analogous to several documented E. coli downstream boxes [Sprengart et al. 1990; Shean and Gottesman 1992]. These considerations suggest that HemE translation might occur primarily from the weak promoter mRNA.

We confirmed this hypothesis by measuring protein synthetic rates from transcripts originating from the two hemE promoters. The results in figure 8B indicate that hemE translation derives primarily from the weak promoter mRNA and that most strong promoter mRNA molecules are not translated. This could be attributable to several factors, including premature termination or inability to access the AUG when the transcript initiates at the strong promoter. Comparison of hemE::lacZ fusion protein synthesis rates from constructs 1 and 2 re-

Figure 8. Translational efficiency of transcripts originating from the strong and weak hemE promoters. [A] Potential RNA structures formed by mRNA initiating from either the hemE strong promoter or the downstream weak promoter. Base-pairing across the 8-mer motifs (numbered 1–4) in the leader mRNA originating from the strong promoter might block ribosome access to the AUG start codon. RNA sequences downstream of hemE AUG [i.e., the downstream box, Cori positions +198 through +186] might align ribosomes on the weak promoter mRNA lacking a S.D. site by base-pairing with C. crescentus 16S rRNA from positions 1498 through 1486 (Ely 1992), as is observed for some E. coli [Sprengart et al. 1990] and bacteriophage λ [Shean and Gottesman 1992] genes. (B) The mRNA originating from the strong hemE promoter is translated inefficiently. C. crescentus strains NA1000 containing constructs 1–5, corresponding to plasmids pGM4001–pGM4005 in Table 3, were pulse-labeled with 35S-methionine, and equal amounts of 35S-labeled protein extracts were immunoprecipitated with anti-β-galactosidase antibody. To aid the comparison of relative protein synthetic rates, the immunoprecipitated proteins were separated on a single polyacrylamide gel, and the resulting bands were quantitated by PhosphorImaging. The indicated numbers show protein synthesis units expressed as PhosphorImaging counts per band obtained after a 24-hr exposure to the PhosphorImaging screen [Molecular Dynamics]. To normalize for transcription termination sequences, we compared constructs with the same lengths of hemE coding sequence, between the HindIII site (-633) and the AUG start codon (constructs 1–4). S.D. is the efficient Shine and Dalgarno site directing lacZ translation; other symbols are as in fig. 1A.
veals that most hemE translation is directed by the weak promoter, because addition of the strong promoter only increases protein synthesis from 3172 to 4130 Miller units. This is a very small increase, considering the relative strengths of these promoters. When the activities of constructs 1 and 2 are compared with that of constructs 3 and 4, it is evident that hemE translation from its native ribosome-binding sites is not very efficient, because a S.D. sequence provided by the lacZ reporter gene increased the rate of protein synthesis ~10-fold. The addition of the strong promoter to the weak promoter in constructs 3 and 4 also provided a disproportionately small increase in lacZ protein synthesis from 34,783 to 47,438 Miller units. Although the strong promoter's contribution to downstream protein synthesis is relatively minor in constructs 2 and 4, its full protein synthetic potential is revealed in construct 5 where the 8-mer motifs are deleted and an efficient S.D. sequence is provided by the lacZ reporter gene. Construct 5 demonstrates that the strong promoter can drive as much as 443,971 units of lacZ protein synthesis, but that in its normal context the strong promoter only contributes ~1000 units [cf. constructs 1 and 2]. Apparently, most transcripts that initiate from the strong hemE promoter do not contribute significantly to hemE gene expression. Presumably this RNA serves a replicative function.

Discussion

The C. crescentus predivisional cell exhibits a striking asymmetry that yields morphologically distinct progeny whose chromosomes have different replicative fates. Although the swarmer cell delays replication, the stalked cell initiates replication of the entire chromosome from a single origin and replication proceeds bidirectionally at a uniform rate until completion (Lott et al. 1987, Dingwall and Shapiro 1989, Marczynski and Shapiro 1992). Apparently, control mechanisms for replication initiation act asymmetrically in the predivisional cell, and we have focused our studies on the cloned C. crescentus origin of replication (Cori). Mutation analysis demonstrated that, in addition to anticipated features such as an AT-rich region and DnaA boxes, Cori contains novel replication elements such as 8-mer and 9-mer motifs that are also essential for replication [Fig. 1]. Interestingly, both 8-mer and 9-mer motifs are clustered in the 5' region of the hemE gene, whose unusual transcription and translation properties are summarized in Figure 9A.

From our analysis, we conclude that replication is coupled to transcription from the strong hemE promoter originating from inside Cori. Seven major experimental results support this conclusion. (1) The 5' region of the hemE gene overlaps the essential Cori replication region [Fig. 1]. (2) Two hemE promoters intimately overlap repeated 8-mer and 9-mer motifs that are essential for replication [Figs. 2 and 3]. The two 9-mer motifs that overlap the −10 and the −35 regions of the strong promoter are also present at three additional places inside Cori where their integrity is essential for replication. (3) Mutations that decrease promoter activity also impair replication [Fig. 3C,D]. (4) Targeted mutagenesis that decreases strong promoter transcription [without changing the overlapping 9-mer motifs] impairs replication, implying that transcription is required for replication [Fig. 3D]. (5) Translation of the HemE protein is significantly under-represented in comparison to the activity of the strong hemE promoter, implying that most transcripts initiating from the strong hemE promoter are not translated and presumably serve an alternative replicative function [Fig. 8B]. (6) There is preferential transcription from the strong hemE promoter in the stalked cell pole of the predivisional cell and in the nascent stalked cell [Figs. 4B, 5A, B, and 6C]. Therefore, transcription from the strong promoter anticipates chromosome replication. (7) When transcription from this strong promoter is impaired so that it is transcribed comparably at both poles [Figs. 5C,D and 6D], the control of replication is lost in the progeny cells [Fig. 7]. We propose that preferential transcription from the strong promoter establishes a polarity in the predivisional cell that generates progeny whose chromosomes have different replicative fates [Fig. 9B].
Polarized transcription in the *C. crescentus* predivisional cell requires a special transcriptional control system. Although most expressed genes are transcribed from both chromosomes in the predivisional cell, four flagellar promoters are known to be transcribed selectively at the swarmer pole (Gober et al. 1991a,b, Gober and Shapiro 1992). We observed that the strong *hemE* promoter decreases transcription at the swarmer pole, whereas stalked pole transcription remains constant during the predivisional cell period (Fig. 5B). This suggests that differential transcription from the strong promoter is attributable either to swarmer pole-directed repression or to stalked pole activation. The promoter deletion in pGM1030 (Fig. 3D), which affects both 9-mers, simultaneously yielded equal transcription at the swarmer and stalked poles (Figs. 5C,D and Fig. 6D) and eliminated normal replication control in the progeny cells (Fig. 7B,D). Therefore, we suggest that a regulatory protein binds differentially to the two 9-mer motifs that overlap the −10 and −35 regions of the strong *hemE* promoters in the predivisional cell. Recent results indicate that a response regulatory element protein that acts at multiple points of the *C. crescentus* cell cycle binds to the 9-mer motifs in the strong *hemE* promoter (G. Marczynski, K. Quon, and L. Shapiro, unpubl.).

The activation of replication by transcription was originally encountered in the *E. coli* λ phage and the *E. coli* chromosomal replication systems (for review, see Baker and Wickner 1992). In both cases, transcription is required for replication, although RNA polymerase probably does not actually synthesize a primer for DNA polymerase (Ogawa et al. 1985; Baker and Kornberg 1988). These systems contrast with plasmid ColE1 (Masukata and Tomizawa 1990) and mitochondrial DNA replication (de Zamaroczy et al. 1984; Schinkel and Tabak 1989; Clayton 1991), where RNA polymerase synthesizes the rate-limiting RNA primer for DNA polymerase. Plasmid ColE1 copy number control is dependent on a complex interplay between the secondary structure of the primer RNA and competition between annealing to an antisense RNA repressor or complementary DNA inside the ColE1 origin (Kues and Stahl 1989). Transcription from the *Cori* strong promoter through the 8-mer cluster blocks HemE translation (Fig. 8B), presumably by forming an RNA secondary structure in the leader sequence that blocks ribosome binding (Fig. 8A). By analogy to ColE1 plasmids, we speculate that this RNA structure has an additional replicative role inside *Cori*. The majority of the base pairs in this structure form by virtue of the 8-mer motifs, and mutations inside the 8-mer cluster impair replication (Fig. 3C). We speculate that the 8-mer motifs are RNA structural elements, whereas the 9-mer motifs are DNA-binding sites for new replication proteins.

We also speculate that the *C. crescentus* *hemE* promoter/origin overlap represents an ancestral counterpart to the mitochondrial RNA-primed origin. Both *C. crescentus* and the ancestral endosymbiont that gave rise to the modern mitochondrion are members of the α-subdivision of purple bacteria (Yang et al. 1985; Woese 1987; Schmidt et al. 1991; Stahl et al. 1992). Promoters like the strong *hemE* promoter, which are not coupled to genetic expression, are characteristic of yeast mitochondrial origins of replication (de Zamaroczy et al. 1984; Schmitt and Clayton 1993). The sequences downstream of the strong *hemE* promoter are 83% GC (Fig. 9A), and our mutations define this GC block as absolutely essential for replication (Fig. 3C). As shown in Figure 9B, we propose that this GC block may clamp the 5′ end of the *hemE* mRNA preferentially at the stalked pole of the predivisional cell, where we have demonstrated that this promoter is transcribed preferentially before cell division. This clamping mechanism and the proximity of the GC block to the RNA start site are directly analogous to mitochondrial replication origins (Schmitt and Clayton 1993). A persistent *hemE* RNA–DNA hybrid might serve as a heritable replication determinant to preferentially stimulate replication in the progeny stalked cell.

The mechanism for stimulating replication may involve establishing a rate-limiting primer (by RNase H cleavage) or destabilizing the adjacent 85% AT region, as implied in Figure 9B. Short RNA/template hybrids (R-loops) located at or near the *E. coli* origin stimulate replication in vitro, apparently by promoting DNA melting under suboptimal conditions (e.g., low supercoiling, or high HU chromatin protein concentration) that would otherwise inhibit the DnaA-dependent strand-separating reaction at the AT-rich region (Baker and Kornberg 1988; Skarstad et al. 1990). The *E. coli* mioC and gidA genes flank the origin, but their promoters are separate from the minimal *E. coli* origin (Oka et al. 1980, 1984). However, their transcription can influence replication significantly under special circumstances (Asai et al. 1990, Lobner-Olesen and Boye 1992). Recently, it has been demonstrated that gidA and mioC transcription is modulated during the *E. coli* cell cycle such that gidA transcription increases while mioC transcription decreases before the initiation of replication (Theisen et al. 1993; Ogawa and Okazaki 1994). Also, and perhaps more similar to our results, transcription from specific promoters inside the *E. coli* origin may cooperate with the DnaA protein to unwind the AT-rich region (Asai et al. 1992). Our observations on transcription within the *C. crescentus* origin of replication imply that it may use general transcriptional control mechanisms to polarize both replication and morphogenesis in the asymmetric predivisional cell. Although, other mechanisms can be proposed, currently we favor the model presented in Figure 9B because it uses established biochemical properties and it makes experimentally testable predictions.

Materials and methods

**Bacterial strains**

*E. coli* strain TG1 was used for routine DNA manipulations. *E. coli* S17-1 (Simon et al. 1983) was used to mobilize pRK290 plasmid derivatives into *C. crescentus* by bacterial conjugation (Ely 1979). *E. coli* strain CJ236 was used to prepare single-strand templates for site directed mutagenesis (Kunkel et al. 1987). *C. crescentus* NA1000 (Evinger and Agabian 1977) was used for...
cell synchrony and transcriptional studies, and its ampicillin-sensitive and recombination-defective derivative, LS108 (Table 3) was used for Cori-plasmid replication studies.

Cell growth, synchrony, protein labeling, and immunoprecipitation

C. crescentus strains were grown in PYE medium (Poindexter 1964) or in M2G medium [Ely and Johnson 1977]. pRK290-derived plasmids and pBluescript II-derived Cori plasmids were maintained in C. crescentus cultures by supplementing the media with 1 μg/ml of tetracycline or with 20 μg/ml of ampicillin, respectively. Cells were synchronized by collecting the more dense swarmer cells from a Ludox (colloidal silica) gradient and returning them to fresh M2G medium (Evinger and Agabian 1977). Typically, 1 liter of M2G cultures OD660 0.5–1.0 was concentrated into 150 ml by centrifugation before applying the Ludox protocol yielding ~50 ml of OD660 = 0.4–0.8 swarmer cells (>95% pure, as judged by light microscopy). Protocols for labeling synchronous C. crescentus cells with [35S]methionine and immunoprecipitating radiolabeled proteins have been described (Gomes and Shapiro 1984).

Transcriptional analysis

Transcription initiation at the hemE promoters was measured using lacZ transcriptional fusions containing translational stop codons in all three frames preceding the lacZ ribosome-binding sites (Yu and Shapiro 1992). This allowed transcription passing through the lacZ gene to be measured indirectly by pulse-labeling 1.0 ml of cells containing the pRK290 lacZ constructs with 0.01 mCi of [35S]methionine (Trans-35S-label, ICN Radiochemicals) for 5 min, and immunoprecipitation with anti-β-galactosidase antibody (Gomes and Shapiro 1984). Polar transcription localization experiments were performed as reported (Gober et al. 1991b), and nascent swarmer cells and stalked cells were isolated by combining the Ludox and differential centrifugation protocols (Gober and Shapiro 1992). β-Galactosidase assays were performed on unsynchronized exponential cultures OD660 = 0.2–0.4 of C. crescentus strain NA1000 containing the pRK290 lacZ reporter plasmids with Cori subclones [Fig. 2, constructs 1–16] as described (Miller 1972). For the primer extension analysis, C. crescentus RNA was prepared from exponentially growing NA1000 cells in M2G media by the hot phenol method (Gomes et al. 1990) and treated with ribonuclease-free DNase I, reextracted with 50:50 phenol/chloroform, ethanol precipitated, and resuspended in sterile water. Approximately 5 μg of this RNA was annealed with ~100,000 cpm of the specified hemE oligonucleotides [Fig. 3A, B], and 5’-end labeled with [γ-32P]ATP (Amersham) and T4 phage polynucleotide kinase. The primer extension reaction was performed with 5 units of avian myeloblastosis virus reverse transcriptase in the presence of actinomycin C, and the products were analyzed on a 6% polyacrylamide sequencing gel alongside dideoxynucleotide sequencing reactions.

Table 3. Strains and plasmids

| Strains/plasmids | Description | Reference/source |
|------------------|-------------|-----------------|
| E. coli         | Δ(lac-pro), supE, thi, hsdS3/F’ proAB, lacI, lacZΔM15 | T.J. Gibson (unpubl.) |
| TG1             |             |                 |
| S17-1           | E. coli 294::RP4-2(Tc::Mu[Km::Tn7]) | Simon et al. (1983) |
| CJ236           | dut1, ung1, thi, relA/pCJ105 | Kunkel et al. (1987) |
| C. crescentus   |             |                 |
| NA1000          | synchronous wild type | Evinger and Agabian (1977) |
| LS108           | NA1000 derivative bla::Tntet, rec–Tn5 | Marczynski and Shapiro (1992) |
| Plasmids        |             |                 |
| pBluescript II  | Amp. ColEI, KS polyn linker | Stratagene |
| pGM946          | wild-type Cori plasmid, 1.7-kb BamHI–HindIII Cori in pBluescript II | this paper |
| pGM1000 series  |             |                 |
| 1001–1030       | pGM946 with deletion/insertions | Table 1 |
| 1031–1038       | pGM946 with targeted mutations | Table 2 |
| 1064            | pGM946 RK2 par. Clal/CcrM sites | Fig. 7 |
| 1065            | pGM1030 RK2 par. Clal/CcrM sites | Fig. 7 |
| pRK290lacZ      | derivative of broad host-range pRK290 (Ditta et al. 1980) with lacZ transcriptional reporter and mp18 poly linker | Yu and Shapiro (1992) |
| pGM2000 series  |             |                 |
| 2001–2016       | pRK290lacZ containing Cori subclones | Fig. 2 |
| 2021–2028       | from pGM946 and pGM1000 series | Fig. 3 |
| pBZ281          | lacZ translational fusion vector | Alley et al. (1991) |
| pGM3901         | hemE::lacZ protein fusion plasmid, BglII to HindIII Cori from pGM946 in pBZ281 | this paper |
| pGM3902         | hemE::lacZ protein fusion plasmid, EcoRI–HindIII Cori from pGM946 in pBZ281 | this paper |
| pGM4000 series  |             |                 |
| 4001            | pGM3901 in EcoRI site of pRK290 | Fig. 8B |
| 4002            | pGM3902 in EcoRI site of pRK290 | Fig. 8B |
| 4003            | BglII–HindIII Cori in pRK290lacZ | Fig. 8B |
| 4004            | EcoRI–HindIII Cori in pRK290lacZ | Fig. 8B |
| 4005            | EcoRI–HindIII (+ 244) Cori from pGM1032 in pRK290lacZ | Fig. 8B |
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DNA preparation, sequencing, mutagenesis, and replication analysis

Alkaline lysis plasmid preparations from *E. coli*, digestions by restriction endonucleases, agarose gel electrophoresis, and Southern blot procedures were performed according to established protocols (Sambrook et al. 1989). DNA sequencing was performed by the dideoxyribonucleotide chain termination method prepared as described (Short et al. 1988). The series of plasmid mutations (Tables 1 and 2) were created by combining (Sanger et al. 1977) using the Sequenase System II kit (U.S. Biochemical). Single-strand DNA pBluescript II phagemids were prepared as described (Short et al. 1988). The series of *ori* plasmid mutations (Fig. 1C, Marczynski et al. 1990) and by in vivo 32P-labeled DNA, as detailed previously (Marczynski and Shapiro 1992).

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References

Alley, M.R.K., S.L. Gomes, W. Alexander, and L. Shapiro. 1991. Genetic analysis of a temporally transcribed chemotaxis gene cluster in Caulobacter crescentus. *Genetics* 129:333-342.

Asai, T., M. Takenami, and M. Imai. 1990. The AT richness and *g yellow* transcription determine the left border of the replication origin of the *E. coli* chromosome. *EMBO J.* 9:4065-4072.

Asai, T., C.-P. Chen, T. Nagata, M. Takenami, and M. Imai. 1992. Transcription in vivo within the replication origin of the *Escherichia coli* chromosome: A mechanism for activating initiation of replication. *Mol. & Gen. Genetics* 231:169-178.

Bachmann, B.J. 1990. Linkage map of *Escherichia coli* K-12, Edition 8. *Microbiol. Rev.* 54:130-197.

Baker, T.A. and A. Kornberg. 1988. Transcriptional activation of initiation of replication from the *E. coli* chromosomal origin: An RNA-DNA hybrid near oriC. *Cell* 55:113-123.

Baker, T.A. and S.H. Wickner. 1992. Genetics and enzymology of DNA replication in *Escherichia coli*. *Annu. Rev. Genet.* 26:447-477.

Brambill, D. and A. Kornberg. 1988a. Duplex opening by DnaA protein at novel sequences in initiation of replication at the origin of the *E. coli* chromosome. *Cell* 52:743-755.

---. 1988b. A model for initiation at origins of DNA replication. *Cell* 54:915-918.

Brun, Y.V., G.T. Marczynski, and L. Shapiro. 1994. The expression of asymmetry during *Caulobacter* cell differentiation. *Annu. Rev. Biochem.* 63:419-450.

Clayton, D.A. 1991. Replication and transcription of vertebrate mitochondrial DNA. *Annu. Rev. Cell Biol.* 7:453-478.

DePamphilis, M.L. 1988. Transcriptional elements as components of eukaryotic origins of DNA replication. *Cell* 52:635-638.

de Zamaroczy, M., G. Faugeron-Fonty, G. Baldacci, R. Goursot, and G. Bernardi. 1984. The ori sequences of the mitochondrial genome of a wild-type yeast strain: Number, location, orientation and structure. *Gene* 32:439-457.

Dingwall, A. and L. Shapiro. 1989. Rate, origin, and bidirectionality of *Caulobacter* chromosome replication as determined by pulsed-field gel electrophoresis. *Proc. Natl. Acad. Sci.* 86:119-123.

Ely, B. 1979. Transfer of drug resistance factors to the dimorphic bacterium *Caulobacter crescentus*. *Genetics* 91:371-380.

---. 1992. DNA sequence of the 3’ end of the *Caulobacter crescentus* 16S rRNA gene. *Nucleic Acids Res.* 20:1423.

Ely, B. and R.C. Johnson. 1977. Generalized transduction in *Caulobacter crescentus*. *Genetics* 87:391-399.

Evinger, M. and N. Agabian. 1977. Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmers cells. *J. Bacteriol.* 132:294-301.

---. 1979. *Caulobacter crescentus* nucleoid: Analysis of sedimentation behavior and protein composition during the cell cycle. *Proc. Natl. Acad. Sci.* 76:175-178.

Gilchrist, A. and J. Smit. 1991. Transformation of freshwater and marine *Caulobacter* by electroporation. *J. Bacteriol.* 173:921-925.

Gober, J.W. and L. Shapiro. 1990. Integration host factor is required for the activation of developmentally regulated genes in *Caulobacter*. *Genes & Dev.* 4:1494-1504.

---. 1992. A developmentally regulated *Caulobacter* Flagellar promoter is activated by 3’ enhancer and IHF binding elements. *Mol. Biol. Cell* 3:913-926.

Gober, J.W., M.R. Alley, and L. Shapiro. 1991a. Positional information during *Caulobacter* cell differentiation. *Curr. Opin. Genet. Dev.* 1:324-329.

---. 1991b. Expression of positional information during cell differentiation in *Caulobacter*. *Cell* 64:381-391.

Gomes, S.L. and L. Shapiro. 1984. Differential expression and position of chemotaxis methylation proteins in *Caulobacter crescentus*. *J. Mol. Biol.* 178:551-568.

Gomes, S.L., I.W. Gober, and L. Shapiro. 1990. Expression of *Caulobacter* heat shock dnaK is developmentally controlled during growth at normal temperatures. *J. Bacteriol.* 172:3051-3059.

Guo, Z. and M.L. DePamphilis. 1992. Specific transcription factors stimulate simian virus 40 and polyomavirus origins of DNA replication. *Mol. Cell. Biol.* 12:2514-2524.

Heffron, F., M. So, and B.J. McCarthy. 1978. In vitro mutagenesis of a circular DNA molecule by using synthetic restriction sites. *Proc. Natl. Acad. Sci.* 75:6012-6016.

Heintz, N.H., L. Daitey, P. Held, and N.H. Heintz. 1992. Eukaryotic replication origins as promoters of bidirectional DNA synthesis. *Trends Genet.* 8:376-381.

Kornberg, A. and T.A. Baker. 1992. DNA replication. *W.H. Freeman*, New York.

Kowalski, D. and M.J. Eddy. 1989. The DNA unwinding element: A novel, cis-acting component that facilitates opening of the *Escherichia coli* replication origin. *EMBO J.* 8:4335-4344.

Kues, U. and U. Stahl. 1989. Replication of plasmids in gram-negative bacteria. *Microbiol. Rev.* 53:491-516.

Kunkel, T.A., J.D. Roberts, and R.A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selec-
Developmentally controlled replication origin

lar cloning and nucleotide sequence of a complete human uroporphyrinogen decarboxylase cDNA. J. Biol. Chem. 261: 9825–9831.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. 74: 5463–5467.

Schinkel, A.H. and H.F. Tabak. 1989. Mitochondrial RNA polymerase: Dual role in transcription and replication. Trends Genet. 5: 149–154.

Schmidt, T.M., E.F. DeLong, and N.R. Pace. 1991. Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. J. Bacteriol. 173: 4371–4378.

Schmitt, M.E. and D.A. Clayton. 1993. Conserved features of yeast and mammalian mitochondrial DNA replication. Curr. Opin. Genet. Dev. 3: 769–774.

Shean, C.S. and M.E. Gottesman. 1992. Translation of the prophage lacI transcript. Cell 70: 513–522.

Shore, J.M., J.M. Fernandez, J.A. Sorge, and W.D. Huse. 1988. AZAP: A bacteriophage λ expression vector with in vivo excision properties. Nucleic Acids Res. 16: 7583–7600.

Simon, R., U. Prieler, and A. Puhler. 1983. A broad host range mobilization system for in vivo genetic engineering: Transposon mutagenesis in gram-negative bacteria. Biotechnology 1: 784–791.

Skarstad, K., T.A. Baker, and A. Kornberg. 1990. Strand separation required for initiation of replication at the chromosomal origin of Coli is facilitated by a distant RNA–DNA hybrid. EMBO J. 9: 2341–2348.

Sprengart, M.L., H.P. Fatseris, and E. Fuchs. 1990. The initiation of translation in E. coli: Apparent base pairing between the 16S rRNA and downstream sequences of the mRNA. Nucleic Acids Res. 18: 1719–1723.

Stahl, D.A., R. Key, B. Flesher, and J. Smit. 1992. The phylogeny of marine and freshwater Caulobacters reflects their habitat. J. Bacteriol. 174: 2193–2198.

Swoboda, U.K., C.S. Dow, and L. Vitkovic. 1982. Nucleoids of Caulobacter crescentus C8B5. J. Gen. Microbiol. 128: 279–288.

Theissen, P.W., J.E. Grimwade, A.C. Leonard, J.A. Bogan, and C.E. Helmstetter. 1993. Correlation of gene transcription with the time of initiation of chromosome replication in Escherichia coli. Mol. Microbiol. 7: 575–584.

Wingrove, J.A., E.K. Mangan, and J.W. Goher. 1993. Spatial and temporal phosphorylation of a transcriptional activator regulates pole-specific gene expression in Caulobacter. Genes & Dev. 7: 979–1992.

Woese, C.R. 1987. Bacterial evolution. Microbiol. Rev. 51: 221–271.

Yang, D., Y. Oyaizu, H. Oyaizu, G.J. Olsen, and C.R. Woese. 1985. Mitochondrial origins. Proc. Natl. Acad. Sci. 82: 4443–4447.

Yoshikawa, H. and N. Ogawasawa. 1991. Structure and function of DnaA and the DnaA-box in eubacteria: Evolutionary relationships of bacterial replication origins. Mol. Microbiol. 5: 2589–2697.

Yu, J. and L. Shapiro. 1992. Early Caulobacter crescentus genes fliL and fliM are required for flagellar gene expression and normal cell division. J. Bacteriol. 174: 3327–3338.

Zweiger, G. and L. Shapiro. 1994. Expression of Caulobacter dnaA as a function of the cell cycle. J. Bacteriol. 176: 401–408.

Zweiger, G., G.T. Marczyński, and L. Shapiro. 1994. A Caulobacter methyltransferase that only functions in the predivisonal cell. J. Mol. Biol. 235: 472–485.
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References

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