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Principal Sigma Subunit of the *Caulobacter crescentus* RNA Polymerase

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We have identified the gene encoding the *Caulobacter crescentus* principal sigma subunit, RpoD. The *rpoD* gene codes for a polypeptide of 653 amino acids with a predicted molecular mass of 72,623 Da (σ^{73}). The *C. crescentus* sigma subunit has extensive amino acid sequence homology with the principal sigma factors of a number of divergent procaryotes. In particular, the segments designated region 2 that are involved in core polymerase binding and promoter recognition were identical among these bacteria despite the fact that the −10 region recognized by the *C. crescentus* σ^{73} differs significantly from that of the other bacteria. Thus, it appears that additional sigma factor regions must be involved in −10 region recognition. This conclusion was strengthened by a heterologous complementation assay in which *C. crescentus* σ^{73} was capable of complementing the *Escherichia coli rpoD285* temperature-sensitive mutant. Furthermore, *C. crescentus* σ^{73} conferred new specificity on the *E. coli* RNA polymerase, allowing the expression of *C. crescentus* promoters in *E. coli*. Thus, the *C. crescentus* σ^{73} appears to have a broader specificity than does the σ^{70} of the enteric bacteria.

Caulobacter crescentus is a dimorphic, gram-negative bacterium with a well-defined cell cycle. During the processes of cell differentiation and cell division, a number of morphological and biochemical changes result in the conversion of a stalked cell into two dissimilar progeny cells. One of the progeny is the same as the parent, a sessile stalked cell which can immediately initiate DNA replication and the process of cell division. The other is a motile swarmer cell which is not competent for DNA replication. Before the swarmer cell can commence DNA replication and the subsequent cell differentiation events, it must lose its flagellum and generate a stalk in the place previously occupied by the flagellum. Once the stalk is formed, the cell initiates DNA replication and proceeds through the cell cycle (for reviews, see references 31 and 22). The cell cycle events resulting in and from cell differentiation are dependent on de novo RNA synthesis (21), suggesting that the regulation of gene expression in this system may be controlled at the level of transcription. The expression of a number of flagellar genes is regulated by an alternative form of the sigma subunit (5, 20) and additional *trans*-acting regulatory factors (23, 25). Thus, modification of the transcriptional apparatus seems to be one way by which differential gene expression occurs.

The purified RNA polymerase of *C. crescentus* is composed of subunits analogous to those of other eubacterial RNA polymerases (3). Furthermore, Amemiya and Shapiro (1) demonstrated that the *C. crescentus* RNA polymerase recognizes the same promoter and terminator signals as the *Escherichia coli* enzyme. However, *C. crescentus* promoters are not recognized by *E. coli* RNA polymerase (1, 35, 40). Furthermore, we have shown that the *C. crescentus* promoter sequences are different from those of the canonical promoter sequence recognized by enteric bacteria (19). These results suggest that the principal sigma factor involved in promoter recognition in *C. crescentus* is likely to have a broader specificity than that of the corresponding enteric sigma factors. We describe here the gene coding for the principal sigma subunit of the *C. crescentus* RNA polymerase and demonstrate that it can confer new specificity on the *E. coli* RNA polymerase.

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MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The bacterial strains and plasmids used in this study are described in Table 1. *C. crescentus* strains were grown in PYE medium (14) at 32°C. Minimal M2 glucose medium was used when cells were prepared for synchronization (2). *E. coli* strains were grown in L broth or 2× YT medium (27). Antibiotic supplements were used in the indicated concentrations for *C. crescentus* (tetracycline, 1 mg/liter; ampicillin, 20 mg/liter; and chloramphenicol, 1 mg/liter) and for *E. coli* strains (tetracycline, 10 mg/liter; ampicillin, 100 mg/liter; chloramphenicol, 10 mg/liter; and kanamycin, 50 mg/liter).

Molecular techniques. Plasmid DNA extraction, DNA digestion, ligation, and transformation of *E. coli* host cells were carried out as described by Sambrook et al. (27). Electroporation was performed as described previously (18). All restriction and modifying enzymes were obtained from New England Biolabs Inc. (Beverly, Mass.), and chemicals were purchased from Sigma (St. Louis, Mo.). DNA sequence analysis was carried out by the dideoxy chain termination method of Sanger et al. (28) with a Sequenase kit (Amersham/United States Biochemical Corporation, Cleveland, Ohio). Nucleotide sequences on both strands were determined. Computer analyses of the DNA sequences were performed with the Genetics Computer Group program (9). The DNA probes used for S1 mapping were gel purified and end labeled by using [γ-^{32}P]ATP and T4 polynucleotide kinase. S1 nuclease mapping was done by the method of Berk and Sharp (4).

Construction of a chromosomal transcriptional fusion. For construction of an *rpoD*::*cat* fusion, a 1.2-kb *Sac*I-*Pst*I fragment carrying the 5′ portion of the *rpoD* gene and the upstream flanking region was inserted in front of the promoterless *cat* gene in pJM2-20 (36). This plasmid is a pUC18 derivative and contains a promoterless *cat* cassette in its *Hin*dIII site. The *rpoD*::*cat* fusion was integrated into the chromosome by introducing the fusion plasmid, pJM198, into the synchronizable *C. crescentus* strain SU206 (*syn-1000 bla-6*) by electroporation. Ampicillin-resistant colonies that arose by homologous recombination and integration of the entire plasmid into the genome were selected and characterized by Southern analysis.

Cell labeling and immunoprecipitation. Synchronized cultures of swarmer cells were prepared by differential centrifugation (2). At 0, 15, 30, 45, 60, 90, 120, and 150 min, 1-ml aliquots were removed and pulse-labeled with 10 μCi of Tran^{35}S label (ICN, Costa Mesa, Calif.) for 10 min at 32°C. Labeled cells were immediately frozen in a dry ice-ethanol bath. These cells were lysed by suspension in wash buffer (0.05 M Tris-HCl [pH 8.0], 0.45 M NaCl, 2% [vol/vol] Triton X-100, containing 2 mg of lysozyme per ml). After 30 min on ice, DNA in the samples was sheared by five passages through a 26 1/2-gauge hypodermic needle and cell extracts were incubated with Staph A cells (Sigma) to eliminate nonspecific binding. After centrifugation, the supernatant was divided into two portions. One portion was incubated with anti-chloramphenicol acetyltransferase antibody (5′-3′ Inc., Boulder, Colo.), and the other portion was incubated with

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Description	Source or reference
Strains		
E. coli		
UQ285	*rpoD285*(Ts) *lacZ4 argG75*	*E. coli* Genetic Stock Center
UQ285(pJM185)	*rpoD285*(Ts) *lacZ4 argG75 rpoD$_{cc}$*	This study
XL1-Blue	*recA1 lac endA1 gyrA96 thi hsdR19 supE44 relA1* (F′ *proAB lacI^q lacZ dlM15* Tn*10*)	Stratagene
C. crescentus		
LS107	*syn-1000 bla-6*	M. R. K. Alley and L. Shapiro
SC4033	*syn-1000 bla-6 rpoD*::*cat rpoD*$^+$	This study
Plasmids		
pJCT200	Cosmid containing the *C. crescentus rpoD* and *ilvD* genes	35
pJM2-20	pUC18 containing a promoterless *cat* gene	36
pJM185	pKS$^+$ containing a 3.0-kb *Sac*I-*Sma*I fragment containing the *rpoD* gene	This study
pJM186	pKS$^+$ containing the 3.0-kb *Sac*I-*Sma*I fragment from pJM185	This study
pJM187	pKS$^+$ containing a 2.9-kb *Pvu*II-*Sma*I fragment from pJM186	This study
pJM198	pJM2-20 containing a 1.2-kb *Sac*I-*PstI rpoD* promoter fragment upstream from the *cat* gene	This study
pLEC2903	Mutated *pleC* promoter containing a −10 region that matches the *C. crescentus* consensus, fused to a *lacZ* reporter gene	19
pKS$^+$	pBluescript II KS$^+$	Stratagene
pSK$^-$	pBluescript II SK$^+$	Stratagene

antiflagellin antibody. One hour later, Staph A cells were added to each sample to precipitate the specific antibody-antigen complexes. After 30 min on ice, the immunocomplexes were collected by centrifugation and the supernatants were discarded. The pellets were washed with wash buffer and analyzed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis and autoradiography.

Nucleotide sequence accession number. The nucleotide sequence reported in Fig. 2 has been deposited in the GenBank database under accession number U35138.

RESULTS

Cloning and sequencing of the *rpoD* gene. During the characterization of a DNA fragment containing the *ilvR* gene, which codes for a LysR-type transcriptional factor (18), a DNA sequence which matches a portion of the DNA sequences of a number of genes encoding σ^{70}-type sigma factors was found. A 450-bp *Bst*EII-*Bam*HI fragment (Fig. 1) containing this portion of the gene was subsequently used as a probe to identify a clone containing the entire coding region for a sigma factor gene. Both a genomic library clone, pJCT200, and the chromosomal DNA from *C. crescentus* were found to contain the same-size DNA fragments upon restriction enzyme digestion and Southern hybridization (data not shown). A 3.0-kb *Sac*I-*Sma*I DNA fragment, which hybridized with the probe, was cloned into the pBluescriptII-SK$^+$ vector (Stratagene, La Jolla, Calif.). The resulting plasmid, pJM185, was used to determine the DNA sequence of the *Sac*I-*Sma*I region (Fig. 2). A

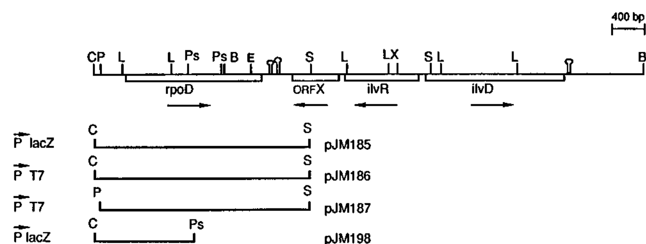

FIG. 1. Genomic and physical organization of the *rpoD* DNA region and plasmids derived from it. The locations of the coding regions are shown by open boxes. Horizontal arrows indicate the direction of the transcription. Relevant restriction enzyme sites are marked as follows: C, *Sac*I; P, *Pvu*II; L, *Sal*I; Ps, *Pst*I; B, *Bam*HI; E, *Bst*EII; S, *Sma*I; and X, *Xho*I. The short arrows indicate the promoters driving transcription of the cloned fragments in plasmid constructs.

1,962-bp open reading frame which could encode a polypeptide with a calculated molecular mass of 72,623 Da was identified. When the deduced amino acid sequence of this gene was compared with amino acid sequences of proteins available in the Swiss-Pro database, a large number of sigma factors from divergent microorganisms showed high levels of similarity. However, the highest levels of amino acid similarity were with those of the principal sigma factors from different microorganisms. Therefore, we designated this open reading frame the *rpoD* gene. An alignment with some of the most similar proteins is shown in Fig. 3. The highest overall levels of identity were with the principal sigma factor of *Agrobacterium tumefaciens* (30) (70%), followed by those of *E. coli* (6) (50%), *Salmonella typhimurium* (11) (50%), *Buchnera aphidicola* (15) (50%), and *Pseudomonas aeruginosa* (34) (50%).

A potential ribosome binding site, GGAG (10), preceded the ATG start codon by 6 nucleotides. Following the TAA translational stop codon at bp 2346, a 20-bp dyad (Fig. 2) that could form a stem-loop structure resembling a *rho*-independent transcription terminator (24) was identified. Another inverted repeat sequence was found further downstream. This structure shows a high degree of sequence similarity with an inverted repeat found at the end of the *C. crescentus flbD* gene (25) and is capable of forming a secondary structure with $\Delta G = -59$ kcal (ca. -250 kJ). This structure could be a transcription termination signal for either *rpoD* or the downstream and divergently transcribed open reading frame X gene.

The predicted amino acid sequence of this sigma factor was found to contain a net charge of −32. Charged residues constituted 31.8% of the total amino acid residues, of which 120 were acidic (E + D) and 88 were basic (R + K) residues. This is one of the characteristic features of sigma factors that contributes to the anomalous migration of these proteins in SDS-polyacrylamide gels (6). For example, the *C. crescentus* purified σ^{73} protein migrates at 102 Da on SDS-polyacrylamide gels (3).

5′ end mapping of the *rpoD* transcript. The 5′ end of the *rpoD* transcript was mapped by nuclease S1 protection assays using two different probes, a 445-bp *Dde*I fragment and a 282-bp *Sst*II fragment. These probes were end labeled at their 5′ ends with [γ-^{32}P]ATP and polynucleotide kinase, annealed to in vivo RNA individually, and then treated with S1 nuclease.

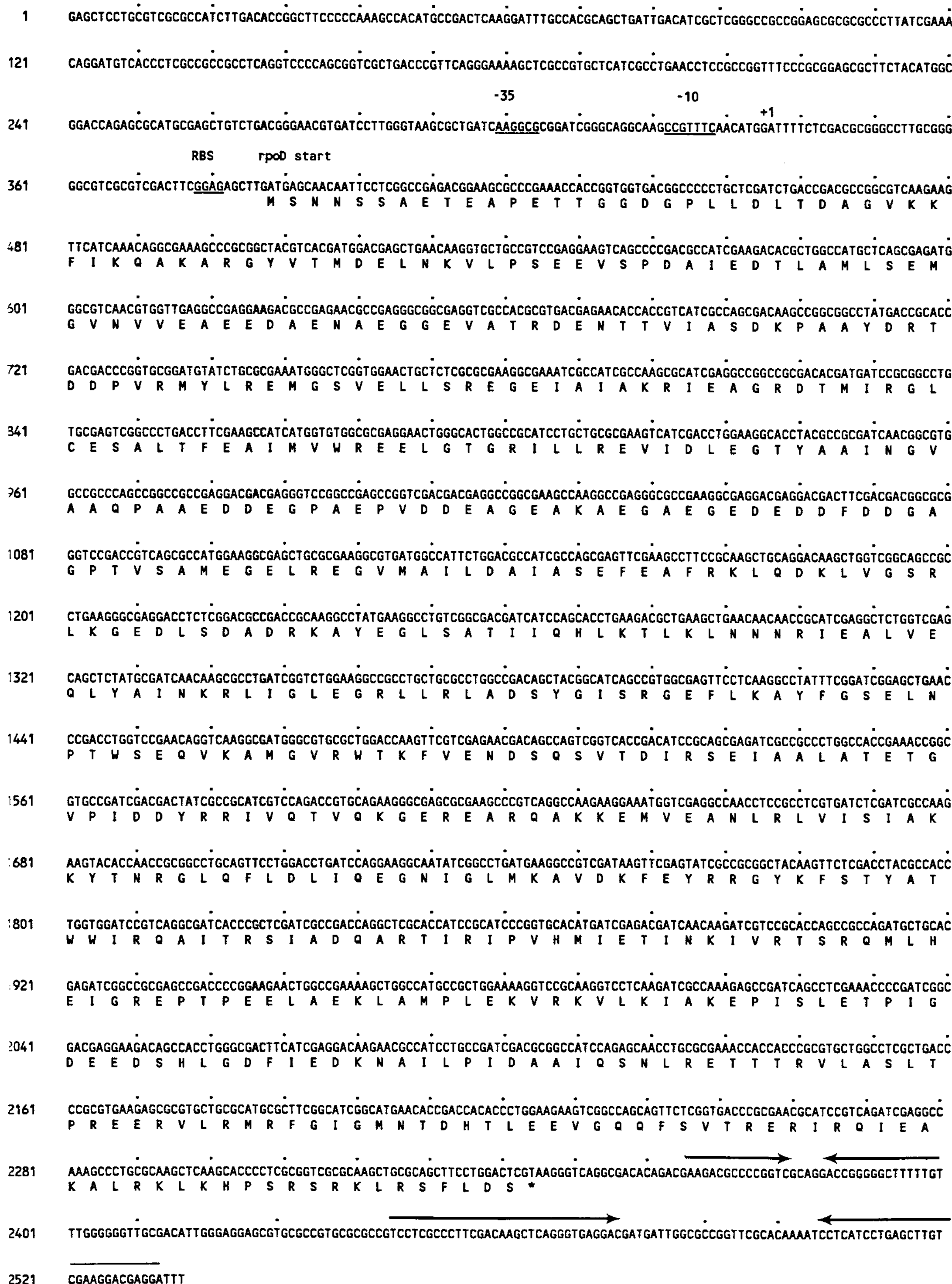

FIG. 2. Nucleotide sequence of the *rpoD* gene. The nucleotide sequence of the *rpoD* gene was determined for both DNA strands. The derived amino acid sequence corresponding to the *C. crescentus* RpoD protein is shown below the DNA sequence. The stop codon is indicated by an asterisk. The ribosome binding site (RBS) upstream from the initiation codon is indicated. The transcription initiation site is marked by +1. The inverted repeat sequences after the coding region are shown by arrows. Numbers on the left refer to the position of the nucleotides.

The 5′ ends of the protected fragments from both experiments coincided at a position 52 nucleotides upstream from the ATG start codon (Fig. 4 and data not shown). In addition to the smaller protected fragment, a band corresponding to a full-size probe was also protected in both S1 experiments.

Expression of the *rpoD* gene during the cell cycle. To demonstrate the time course of *rpoD* gene expression during the cell cycle, a 1.2-kb *Sac*I-*Pst*I fragment containing the promoter (Fig. 1) was placed 5′ to a promoterless *cat* gene in plasmid pJM2-20 (36). The resulting plasmid, pJM198 (Fig. 5A), was

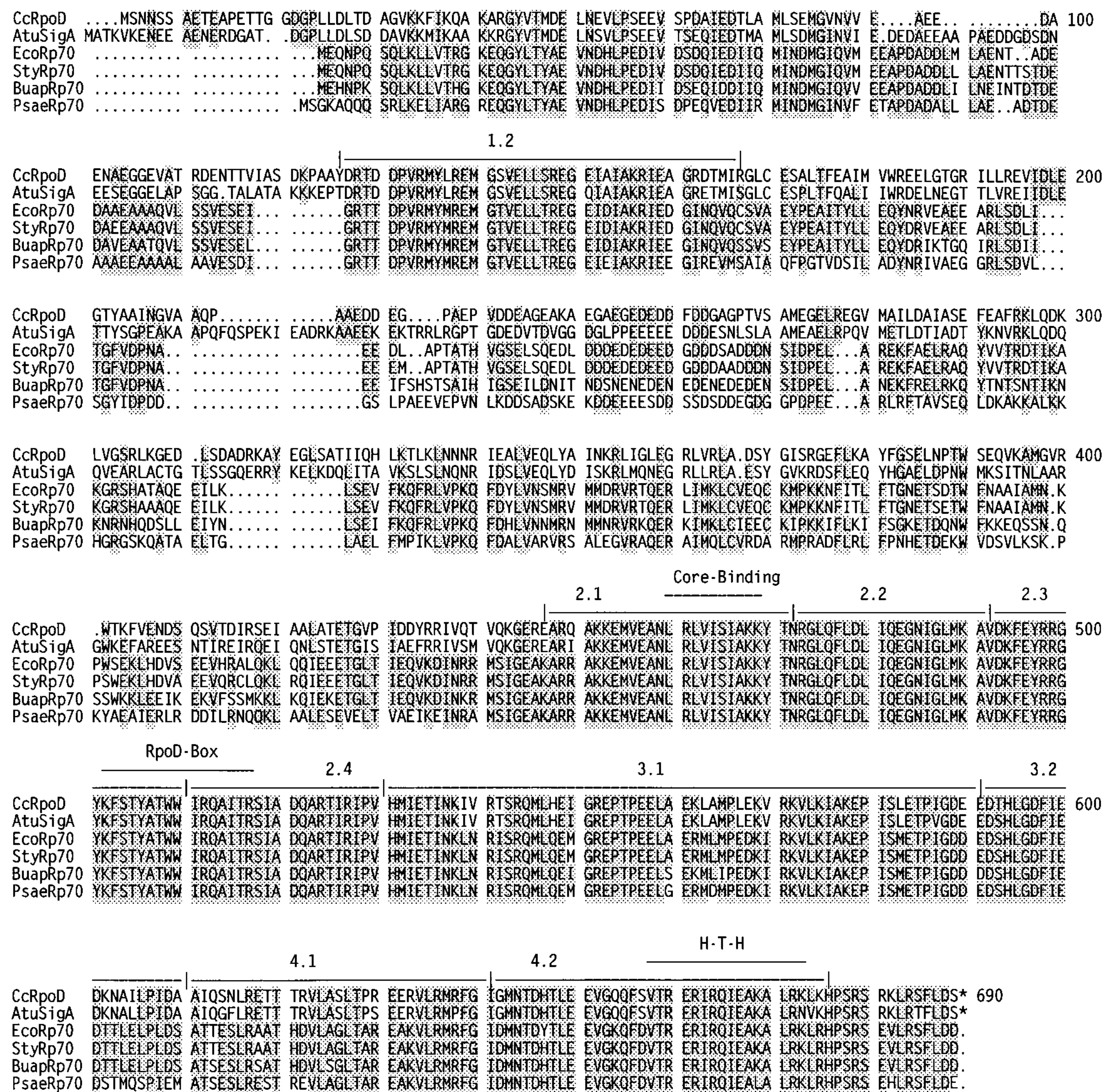

FIG. 3. Amino acid sequence comparison of *C. crescentus* RpoD with the homologous proteins. Amino acid sequence alignment was done by using the Pileup program of the Genetics Computer Group package (9). Gaps introduced to maximize the alignment are shown with dotted lines. The amino acid residues conserved at a given position among all sigma factors listed are shaded. Abbreviations are as follows: CcRpoD, *C. crescentus* RpoD; AtuSigA, *A. tumefaciens* SigA; EcoRp70, *E. coli* RpoD; StyRp70, *S. typhimurium* RpoD; BuapRp70, *B. aphidicola* RpoD; PsaeRp70, *P. aeruginosa* SigD. Numbers at the end of each amino acid sequence refer to total numbers of amino acid residues in that protein. The conserved regions and known functions attributed to each subregion are indicated. The putative helix-turn-helix (H-T-H) motif which may be involved in binding to the −35 region is indicated.

introduced into *C. crescentus* SU206. Since plasmids with a ColE1 origin cannot replicate in *C. crescentus*, Amp^r survivors should result only from the integration of the entire plasmid into the chromosome by homologous recombination. One Amp^r colony was chosen, and integration at the homologous region was confirmed by Southern hybridization (data not shown). The *rpoD*::*cat* integration strain, SC4033, contained a transcriptional fusion between the *rpoD* gene promoter region and the *cat* gene, in addition to an intact copy of the *rpoD* gene (Fig. 5A). The expression of the *rpoD*::*cat* fusion was examined by immunoprecipitation of pulse-labeled chloramphenicol acetyltransferase in a synchronized culture. The *rpoD*::*cat* fusion was expressed throughout the cell cycle (Fig. 5B) in contrast to the control experiment, in which flagellin expression was cell cycle dependent (Fig. 5C). Thus, expression of *rpoD* does not appear to be subject to cell cycle regulation.

***C. crescentus* σ^{73} protein is functionally compatible with the *E. coli* RNA polymerase.** The high degree of amino acid sequence similarity between the *C. crescentus* σ^{73} and *E. coli* σ^{70} prompted us to ask whether the *C. crescentus rpoD* gene can complement an *E. coli rpoD* conditional mutant. *E. coli* UQ285 contains a small, in-frame deletion in the *rpoD* gene that confers a temperature-sensitive growth phenotype because the mutant σ^{70} is rapidly degraded at high temperature (16, 41). To examine the effects of the *C. crescentus* σ^{73} on this mutant strain, UQ285 was transformed with plasmids pJM185, pJM186, pJM187 (Fig. 1), and pBluescriptII-SK$^+$. Both pJM185 and pJM186 harbor the 3.0-kb *Sac*I-*Sma*I fragment (Fig. 1). However, in pJM185, the *rpoD* gene is transcribed via the *E. coli lacZ* gene promoter, while in pJM186 it is located downstream from the T7 promoter, which is not recognized by *E. coli* RNA polymerase. Transformants were selected on L broth-ampicil-

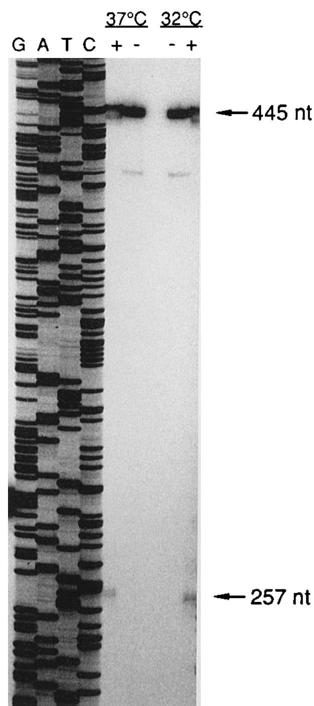

FIG. 4. 5′ end mapping of the *rpoD* transcripts. Total cellular RNA was prepared from the synchronizable *C. crescentus* mutant, SU206 (*syn-1000 bla-6*). In lanes labeled "+", the 5′-end-labeled probe, the 445-bp *Dde*I fragment, was hybridized to 20 μg of RNA at the indicated temperatures and subjected to S1 nuclease digestion. As a control, lanes labeled "−" were subjected to S1 nuclease digestion with no prior hybridization. The molecular size markers (G, A, T, C) were the sequencing reaction samples generated by using BE163, complementary to positions 414 to 432 (Fig. 2) as primer and pJM185 DNA template. The sizes of protected fragments are indicated.

lin plates at 32°C and screened at 42°C. Only colonies carrying plasmid pJM185 were viable at 42°C, while none of the control transformants grew at this temperature. Thus, the *C. crescentus* σ^{73} can functionally replace the defective *E. coli* σ^{70} at the elevated temperature. A UQ235(pJM185) transformant was purified, and its growth rate was compared with that of the untransformed mutant. At 32°C, UQ285(pJM185) should contain both σ^{70} and σ^{73} since the *C. crescentus rpoD* gene is driven by the *lacZ* promoter. Growth curves indicated that it had the same growth rate (48 min) as the control strain, UQ285. This result suggests that the presence of σ^{73} does not interfere with the *E. coli* transcriptional machinery in a way that would disrupt transcription. At 41°C, UQ285 had a 125-min doubling time and UQ285(pJM185) had an 88-min doubling time, indicating that the *C. crescentus* σ^{73} stimulates growth at this partially restrictive temperature. At 43°C, only the UQ285(pJM185) strain was able to grow (doubling time was 140 min). Taken together, these results indicate that the *C. crescentus* σ^{73} can recognize *E. coli* promoter sequences and can provide a functional replacement for the native σ^{70}.

***C. crescentus* σ^{73} confers new specificity on *E. coli* RNA polymerase.** If *C. crescentus* σ^{73} can replace the *E. coli* σ^{70} and express *E. coli* promoters, why is the reverse not true? Why do *C. crescentus* promoters not function properly in *E. coli*? One explanation which is certainly true for some *C. crescentus* genes is that a *trans*-acting factor(s) required for transcription is not present in *E. coli*. Alternatively, despite functional and primary structural similarities among these sigma factors, σ^{73} may have a broader specificity so that it could recognize the *C. crescentus* promoters as well as *E. coli* promoters. In support of this idea, previous experiments have demonstrated that *E. coli* genes are expressed in *C. crescentus* (32, 35, 40).

To examine whether a heterologous holoenzyme containing the *E. coli* core RNA polymerase and the *C. crescentus* σ^{73} would be capable of transcribing *C. crescentus* genes, *E. coli* UQ285 and UQ285(pJM185) were transformed with a *pleC*::*lacZ* fusion plasmid, pLEC2903 (19). The expression of the reporter gene in pLEC2903 is driven by a mutated *C. crescentus* promoter that has little activity in *E. coli* but is expressed well in *C. crescentus* (19). UQ285(pJM185) transformants containing the *C. crescentus rpoD* gene resulted in blue colonies on the indicator plates (L broth–ampicillin–tetracycline–X-Gal [5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside]–IPTG [isopropyl-β-D-thiogalactopyranoside] plates), while the control UQ285 transformant cells remained colorless. β-Galactosidase assays performed after purification of the transformants showed that *pleC* expression was increased more than threefold in the presence of the *C. crescentus rpoD* gene (Table 2). Therefore, these results demonstrate that changing the σ subunit of *E. coli* RNA polymerase changes the promoter recognition capability of the holoenzyme and confers upon the *E. coli* cells the ability to recognize *C. crescentus* promoters.

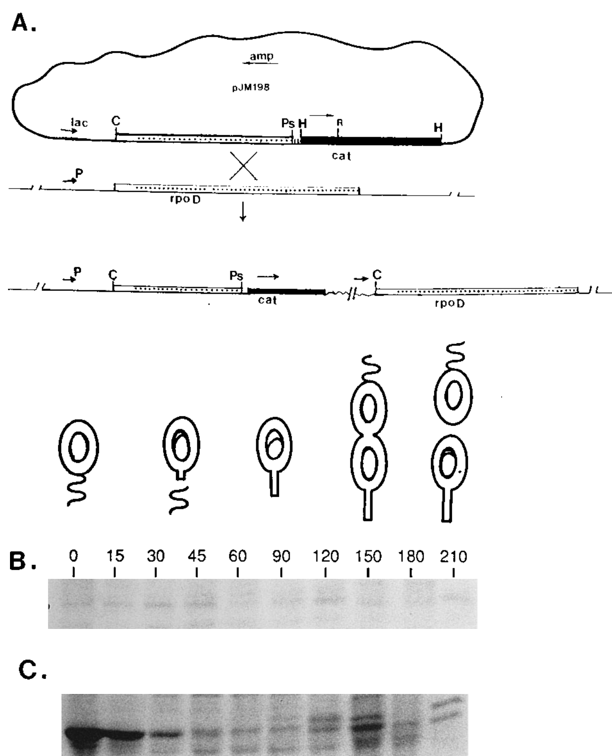

FIG. 5. The expression of chloramphenicol acetyltransferase from a chromosomal *rpoD*::*cat* transcriptional fusion. (A) Schematic drawing of the *rpoD*::*cat* construction. Plasmid pJM198 is shown to have recombined with the chromosomal copy of the *rpoD* gene to generate strain SC4033. (B) Expression of the *rpoD*-driven *cat* gene. Synchronized swarmer cells were isolated and allowed to proceed through the cell cycle. At the indicated time points (in minutes), 1-ml samples were removed, pulse-labeled with Tran^{35}S label (ICN), and processed for immunoprecipitations as described in Materials and Methods. Drawings depict the stage of the cell cycle for each time point. (C) Expression of the flagellin genes as a control for synchrony and progression through the cell cycle.

TABLE 2. Expression of *C. crescentus pleC* promoter in *E. coli*

Strain	Relevant genotype	β-Galactosidase activity[a]
UQ285	*E. coli rpoD*(Ts)	6
UQ285(pJM185)	*E. coli rpoD*(Ts), *C. crescentus rpoD*	5
UQ285(pLEC2903)	*E. coli rpoD*(Ts), *pleC::lacZ*	12
UQ285(pJM185) (pLEC2903)	*E. coli rpoD*(Ts), *C. crescentus rpoD*, *C. crescentus*, *pleC::lacZ*	40

[a] β-Galactosidase activity was measured in cells grown at 33°C and is expressed in Miller units. Each experiment was performed a minimum of two times, and standard deviations were less than 15%.

DISCUSSION

The procaryotic RNA polymerase is composed of four different polypeptides: α, β, β′, and σ. The core enzyme, which has the subunit structure of $\alpha_2\beta\beta'$ in almost all RNA polymerases (cyanobacteria have an extra subunit [29]), is a nonspecific DNA-binding enzyme. The holoenzyme is formed by the association of one of the several species of σ subunits with the core enzyme. Replacement of σ subunits with one another on the core enzyme results in the functional differentiation of the RNA polymerase core enzyme. This, in turn, confers sequence-specific DNA recognition on the holoenzyme, resulting in enhanced interactions with specific promoter sequences. The holoenzyme containing the principal sigma subunit is responsible for transcription of almost all of the genes during the exponential growth phase of a bacterial culture.

In this study, we have identified a gene, *rpoD*, which encodes a sigma factor from *C. crescentus*. On the basis of the following criteria, we concluded that this sigma factor is the principal sigma factor for *C. crescentus*. (i) There is extensive amino acid sequence similarity between the *C. crescentus* σ factor and principal σ factors of other bacteria. (ii) Immunoprecipitation experiments with the *rpoD* transcriptional fusion protein indicated that this protein was present throughout the cell cycle. (iii) In a heterologous complementation assay, the *C. crescentus rpoD* gene was capable of complementing the *E. coli* temperature-sensitive mutant *rpoD285*. (iv) Gene disruption experiments involving the cloned *C. crescentus rpoD* gene were unsuccessful (data not shown), suggesting that the expression of the *rpoD* gene is essential for cell viability. (v) In in vivo experiments, a heterologous RNA polymerase holoenzyme composed of the *C. crescentus* σ^{73} and the *E. coli* core polymerase was able to recognize *C. crescentus* promoters thought to be expressed by holoenzyme containing the principal sigma factor. Taken together, these data present strong evidence that the *rpoD* gene described in this paper encodes the principal sigma factor for *C. crescentus.*

We determined the nucleotide sequence of the entire 2.9-kb DNA region surrounding the *rpoD* gene. Nucleotide sequences 380 bp upstream from the *rpoD* gene failed to reveal any open reading frame conforming to the normal pattern of *C. crescentus* codon usage. In *E. coli* (7, 33) and *Bacillus subtilis* (39), the *rpoD* gene is cotranscribed with the upstream *dnaG* genes. Thus, the gene organization in *C. crescentus* appears to differ from that of the other two bacteria. A sequence similar to a *rho*-independent transcription termination signal was found downstream from the TAA translation stop codon.

The alignment of amino acid sequences of sigma factors from different bacteria has identified four highly conserved regions (13) with further division of each region into functional subregions (17). Of these regions, region 1 is present only in the principal sigma factors and is the least conserved region. The function of this region has not been determined. Region 2 is the most conserved among all sigma factors and is considered to be involved in core binding and recognition of the −10 region of promoter sequences (8, 12, 38). The amino acids encoded by this region were almost identical between *C. crescentus* RpoD and all other sigma factors shown in Fig. 3. This was surprising since the *C. crescentus* promoter sequences recognized by RpoD differ from those used by several of the other microorganisms (19). The −35 region consensus sequence recognized by *C. crescentus* RpoD is identical to that recognized by the enteric RpoD except at position 6. This difference is minor since both sequences are recognized by both RpoD subunits. On the other hand, there appear to be major differences in the −10 region consensus sequences. The *C. crescentus* −10 consensus is GCTANAWC while that of the enteric bacteria is TATAAT. The flanking Cs in the *C. crescentus* −10 consensus are highly conserved and may be the primary reason for the observation that *C. crescentus* biosynthetic promoters are not expressed in *E. coli* (35, 40). The amino acid sequence identity between the *C. crescentus* and *E. coli* proteins in region 2, combined with the lack of promoter compatibility between these microorganisms, suggests that additional sequences may be involved in −10 recognition. Region 3 is highly conserved among the principal sigma factors but is absent or weakly conserved among alternative sigma factors. The function of this region also is not fully understood. Region 4 is highly conserved and contains a helix-turn-helix DNA binding motif. This region participates in binding to −35 promoter sequences (17). The only major difference between *C. crescentus* RpoD and other sigma factors, other than the amino acid differences in the nonconserved region between regions 1 and 2, was the presence of a stretch of 20 extra amino acids at the N terminus of the *C. crescentus* polypeptide. This region was absent in all sigma factors in Fig. 3, except for *A. tumefaciens*, which contained 24 extra amino acids at its N terminus. Whether this region has a functional role in modifying the sigma factor in a way that enables it to recognize specific DNA sequences pertaining to *C. crescentus* promoters remains to be elucidated.

In *E. coli*, a heat shock promoter located in the coding region of the *dnaG* gene is responsible for induction of *rpoD* transcription during a temperature upshift (37). Unlike *E. coli rpoD*, the *C. crescentus rpoD* gene was not induced in response to heat shock (data not shown and reference 26). The expression of the *sigA* gene of *A. tumefaciens* is also not activated by heat shock (30).

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