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# Isolation, Identification, and Transcriptional Specificity of the Heat Shock Sigma Factor $\sigma^{32}$ from *Caulobacter crescentus*

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**We report the identification of the *Caulobacter crescentus* heat shock factor $\sigma^{32}$ as a 34-kDa protein that copurifies with the RNA polymerase holoenzyme. The N-terminal amino acid sequence of this protein was determined and used to design a degenerate oligonucleotide as a probe to identify the corresponding gene, *rpoH*, which encodes a predicted protein with a molecular mass of 33,659 Da. The amino acid sequence of this protein is similar to those of known bacterial heat shock sigma factors of *Escherichia coli* (41% identity), *Pseudomonas aeruginosa* (40% identity), and *Citrobacter freundii* (38% identity). The isolated *C. crescentus* gene complements the growth defect of an *E. coli rpoH* deletion strain at 37°C, and Western blot (immunoblot) analysis confirmed that the gene product is related to the *E. coli* $\sigma^{32}$ protein. The purified RpoH protein in the presence of RNA polymerase core enzyme specifically recognizes the heat shock-regulated promoter P1 of the *C. crescentus dnaK* gene, and base pair substitutions in either the −10 or −35 region of this promoter abolish transcription. S1 nuclease mapping indicates that *rpoH* transcripts originate from two promoters, P1 and P2, under the normal growth conditions. The P2 promoter is similar to the $\sigma^{32}$ promoter consensus, and the P2-specific transcript increases dramatically during heat shock, while the P1-specific transcript remains relatively constant. These results suggest that although the structure and function of *C. crescentus* $\sigma^{32}$ appear to be very similar to those of its *E. coli* counterpart, the *C. crescentus rpoH* gene contains a novel promoter structure and may be positively autoregulated in response to environmental stress.**

The *Caulobacter crescentus* cell cycle generates two different daughter cells: a motile swarmer cell which possesses a single polar flagellum, and a nonmotile stalked cell. The progeny swarmer and stalked cell types differ not only in morphology and motility but also in their programs of gene expression and capacities to reinitiate DNA replication. Formation of the new swarmer cell results from a series of discrete morphogenic events during the cell cycle that occur at one pole of the dividing cell (reviewed in references 8 and 38). This developmental sequence depends on completion of successive cell cycle checkpoints, and there is now evidence that the regulation of cell division and some developmental events is mediated by two-component signal transduction pathways (reviewed in reference 27).

Many developmental events in *C. crescentus* are regulated at the level of transcription initiation. The specificity of promoter recognition is conferred by the sigma subunit of RNA polymerase (RNAP), which binds to the core RNAP (E) containing β, β′, and $\alpha_2$. In eubacterial systems examined to date, multiple sigma factors have been identified, each of which programs RNAP to recognize a distinct promoter sequence. Although the predominant sigma subunit ($\sigma^{70}$ in gram-negative bacteria) directs expression of the bulk of the genome, highly specialized genes involved in physiological and developmental processes are coordinately governed by secondary sigma factor subunits. The appearance and/or activation of these novel sigma factors can reprogram promoter specificity and thus dedicate a portion of the RNAP population to transcribe a selected set of genes (reviewed in reference 21).

RNAP holoenzyme carrying a secondary σ subunit has been shown to play a crucial role in flagellum biosynthesis in *C. crescentus*. Work from this laboratory and others has characterized cell cycle-regulated $\sigma^{54}$-dependent promoters (13, 32, 39), the *rpoN* gene (1, 9), and the transcription activator FlbD (5, 43) that are required for expression of class III and class IV genes at the bottom of the flagellar gene hierarchy. To further explore the biochemistry of the *C. crescentus* RNAP and the role of sigma factor specificity, we have now isolated the $\sigma^{32}$ protein from purified RNAP holoenzyme and cloned the corresponding *rpoH* gene. Heat shock sigma factors have been identified in *Escherichia coli* (19, 26, 47), *Citrobacter freundii* (17), and *Pseudomonas aeruginosa* (6, 33). The best studied of these proteins is *E. coli* $\sigma^{32}$, which is encoded by the *rpoH* (*htpR*, *hin*) gene and plays a central role in the induction of heat shock proteins in response to high temperature or other stress conditions (reviewed in references 10, 29, and 46). When *E. coli* cells are transferred to temperatures higher than 40°C, complex changes in cellular structure, protein composition, and patterns of gene expression occur. The most dramatic response is the increased synthesis of the heat shock proteins. This ubiquitous cellular response is regulated at the level of transcription and requires RNAP containing the $\sigma^{32}$ subunit (37). After transfer to a high growth temperature, there is a short-lived increase of ca. 20-fold in the amount of $\sigma^{32}$, resulting in increased transcription initiation from the $\sigma^{32}$-dependent promoters of heat shock-responsive genes. After 20 to 30 min at the higher temperature, the amount of $\sigma^{32}$ decreases and expression of the heat shock genes declines to three to five times the level before the shift to a high temperature (46).

Little is known about heat shock response and/or other stress responses in *C. crescentus*, although four potential heat shock proteins have been reported (40). Three of them were shown to be immunologically related to the *E. coli* heat shock proteins GroEL, Lon, and DnaK, respectively. A fourth heat shock protein of ca. 37 kDa was also observed and may be related to the $\sigma^{32}$ protein described in this report. In addition,

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the *C. crescentus dnaK* gene and a portion of the *dnaJ* gene encoding the heat shock proteins DnaK and DnaJ have been isolated and sequenced (18). Expression of the *C. crescentus dnaK* gene is temporally regulated during normal cell division from a $\sigma^{70}$-like promoter, P2, and it is induced in responding to heat shock from a $\sigma^{32}$-like promoter, P1 (4, 18).

In this study, we describe the identification and purification of the *C. crescentus* heat shock sigma factor and present evidence demonstrating that this sigma factor specifically recognizes the $\sigma^{32}$-like promoter of the *dnaK* gene in vitro. We have also cloned the *rpoH* gene by using a degenerate DNA probe based on the N-terminal amino acid sequence of the $\sigma^{32}$ protein and demonstrated that in vivo, it is transcribed from two promoters, P1 and P2. The P2 promoter aligns with the $\sigma^{32}$ consensus sequence, and the P2-specific transcript increases dramatically during heat shock. These results suggest that the *C. crescentus* $\sigma^{32}$ may be subject to a novel mechanism of positive autoregulation.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and media.** *E. coli* DH5α was used in this study for propagating plasmids and grown on ML medium supplemented with ampicillin (100 μg/ml) or tetracycline (10 μg/ml) as necessary. *E. coli* KY1608 (gift from T. Yura, HSP Research Institute, Kyoto Research Park, Kyoto, Japan), a nonlysogenic version of KY1612 described by Zhou et al. (48), was grown at 20°C on ML medium supplemented with 15 μg of kanamycin. *C. crescentus* CB15 was grown in either peptone-yeast extract medium or M2 minimal glucose medium (14). pBluescript (Stratagene Cloning System) was used in this study for subcloning and sequencing experiments.

**Isolation and purification of a 34-kDa RNAP subunit from *C. crescentus*.** Cells of *C. crescentus* CB15 grown at 30°C were harvested and used for purification of RNAP. The RNAP core enzyme and holoenzymes were isolated and purified as described elsewhere (45). Samples of RNAP holoenzyme were applied to sodium dodecyl sulfate (SDS)-polyacrylamide gels (10% acrylamide), and after staining with KCl, the gel was sectioned into eight slices corresponding to the positions of visible proteins. Proteins were eluted from the gel slices, freed of SDS by acetone precipitation, dissolved in 6 M guanidine-HCl, and renatured as described by Hager and Burgess (20). The eluted proteins were subsequently combined with *C. crescentus* RNAP core enzyme and tested for sigma factor activities in in vitro transcription assays using different promoters as templates (44). A 34-kDa protein containing $\sigma^{32}$ activity was identified by in vitro transcription assays using the *dnaK* promoter as the template and further purified by the method of Hager and Burgess (20). The 25-amino-acid sequence from N terminus of the purified 34-kDa protein was determined as AVNSLSVMSPDGGLSRYLTEIRKFP at the Princeton SynSeq facility. A degenerate oligonucleotide (OY1; CGCTA[C/T]CT[G/C]AC[G/C]GA[A/G]ATCCG[C/G]AAGTTCCC) was synthesized on the basis of the 10-amino-acid residue sequence RYLTEIRKFP and used as a probe to identify the corresponding gene.

**Isolation and identification of the gene encoding the 34-kDa RNAP subunit.** A *C. crescentus* genomic library in cosmid pLAFR was hybridized in situ by using the degenerate deoxyoligonucleotide OY1. One of the hybridizing colonies was further analyzed with restriction endonucleases and by Southern hybridization to the same probe. Two hybridized fragments, a 1.8-kb *Eco*RI fragment and a 1.6-kb *Pst*I fragment, were identified and subsequently subcloned into pBluescript to yield two plasmids, pJW220 and pJW222, respectively (see Fig. 3). These two plasmids were sequenced by using Sequenase 2.0 (United States Biochemical) and 7-deaza-dGTP in place of dGTP. Sequence analysis was carried out with the Genetics Computer Group package, and searches for sequence homology to the translated gene product were carried out against sequence databases by using the TFASTA program from the Genetics Computer Group.

**Overexpression and purification of *C. crescentus* $\sigma^{32}$ factor.** The *Xba*I-*Hin*dIII fragment from pJW222 was subcloned into *Xba*I and *Hin*dIII sites of mutagenesis vector pALTER-1 (Promega) to generate pJW230. An *Nde*I site was introduced into the first codon of the *rpoH* gene in plasmid pJW230 by site-directed mutagenesis. The *Nde*I-*Hin*dIII fragment from plasmid pJW231 was subcloned into the *Nde*I and *Hin*dIII sites in expression vector pRSET(A) to generate plasmid pJW234. Finally, the *Pst*I-*Hin*dIII fragment containing the remainder of the *rpoH* open reading frame was subcloned from pJW220 into the *Pst*I and *Hin*dIII sites of pJW234. This yielded plasmid pJW235, which carries the entire open reading frame of the *rpoH* gene translationally fused to the first codon of the T7 gene 10 from bacteriophage T7, with expression of the fusion gene under control of the T7 gene 10 promoter.

Plasmid pJW235 was transformed into *E. coli* BL21(DE3) to overproduce $\sigma^{32}$ protein. Cells were grown in ML liquid medium containing ampicillin (100 μg/ml) at 37°C overnight. Two liters of fresh medium was inoculated with 20 ml of the overnight cultures and then incubated at 37°C. When the optical density at 600 nm reached ca. 0.3, the culture was induced with IPTG (isopropyl-β-D-thiogalactopyranoside) at a final concentration of 0.5 mM.

Induced cells were harvested and washed with TE buffer (10 mM Tris-HCl [pH 8.0], 1 mM EDTA). The cells were disrupted with lysis buffer containing lysozyme and deoxycholate. Lysates were precipitated with 24 g of ammonium sulfate per 100 ml, dialyzed into TGED buffer (20 mM Tris-HCl [pH 7.9], 5% glycerol, 0.1 mM EDTA, 0.1 mM dithiothreitol), and chromatographed on a DEAE-cellulose column. The column was developed with a linear salt gradient from 50 to 600 mM NaCl (see Fig. 6). The fractions containing the $\sigma^{32}$ protein were pooled, loaded onto a heparin-Sepharose column, and eluted with a linear salt gradient from 50 to 600 mM NaCl. Fractions containing $\sigma^{32}$ were pooled and dialyzed against stock buffer (TGED in 50% glycerol) and then stored at −80°C. This procedure yielded $\sigma^{32}$ protein that was greater than 95% pure, as judged by Coomassie blue staining of SDS-polyacrylamide gels. Western blotting (immunoblotting) with an antibody against *E. coli* $\sigma^{32}$ was performed as described previously (24).

**In vitro transcription assays.** The formation of open complexes at the $\sigma^{32}$-dependent promoters from the *C. crescentus dnaK* gene was measured in single-cycle runoff transcription assays. The E$\sigma^{32}$ holoenzyme was reconstituted on ice for 10 min by addition of the purified *C. crescentus* $\sigma^{32}$ protein to the purified *C. crescentus* RNAP core enzyme (final concentration of 1 μM). Transcription-competent open complexes were formed at 37°C for 15 min in 50 μl of transcription buffer (50 mM Tris-HCl [pH 7.8], 100 mM NaCl, 1 mM dithiothreitol, 200 μg of bovine serum albumin per ml, 0.1 mM EDTA, 10 mM $MgCl_2$) containing 100 μM each ATP, CTP, and GTP and 5 nM linear DNA template. Elongation of initiated transcript was performed by adding 5 μCi of [α-$^{32}$P]UTP, 10 μM UTP, and 0.5 mg heparin per ml, and the mixture was incubated at 37°C for 10 min. The reactions were stopped by the addition of an equal volume of stop solution, and the transcripts were precipitated with ethanol. The samples were dissolved in 10 μl of sample buffer and run on 7 M urea-polyacrylamide gels with end-labeled *Sau*3A fragments of pUC18 as DNA size markers.

**S1 nuclease protection analysis.** The *Xba*I-*Hin*dIII fragment from plasmid pJW222 was subcloned into the *Xba*I and *Hin*dIII sites of M13mp18. Single-stranded DNA made from this plasmid was used as the template for extension of an oligonucleotide primer, 5′-CGAGGCGCAGGTGGCTGG-3′, by T4 DNA polymerase. The extended duplex DNA containing the labeled strain was digested with *Xba*I, and the 650-bp single-stranded probe was purified by electrophoresis through 7 M urea-polyacrylamide gels. The corresponding fragment was electroeluted from the gel and used as the probe in S1 nuclease mapping (7). The total mRNAs isolated from *C. crescentus* grown at 30°C were hybridized to the labeled probe and then treated with 400 U of S1 nuclease. The protected RNA fragments were subjected to electrophoresis on a sequencing gel containing 7 M urea.

**Nucleotide sequence accession number.** The *rpoH* sequence has been deposited in the GenBank database under accession number U39791.

## RESULTS

**Identification of $\sigma^{32}$ in RNAP preparations from *C. crescentus*.** In our study of the purified *C. crescentus* RNAP, we focused on a prominent protein at ca. 34 kDa on SDS-polyacrylamide gels. This protein copurified with the RNAP holoenzyme (Fig. 1A, lane 2) but not with the RNAP core (lane 1; see Materials and Methods) (45). To determine if the 34-kDa protein behaves as a sigma factor, we eluted the protein from gels and examined its ability to stimulate transcription in vitro. In a reconstituted transcription system using the *C. crescentus dnaK* gene as the template (Materials and Methods), the purified protein stimulated the appearance of a labeled transcript of the size expected if initiation had occurred from the $\sigma^{32}$-dependent promoter, but it failed to recognize other promoters, including $\sigma^{70}$- and $\sigma^{54}$-dependent promoters (44). These results suggest that the 34-kDa protein is a $\sigma^{32}$ homolog. This conclusion is supported by results of Western blot analysis, which showed that an anti-*E. coli* $\sigma^{32}$ antibody recognizes the 34-kDa protein in the RNAP holoenzyme (Fig. 1B, lane 2) but does not react with proteins in the RNAP core enzyme preparation (lane 1).

**Isolation and sequencing of the *C. crescentus rpoH* gene.** To identify and isolate the *C. crescentus* gene encoding the $\sigma^{32}$ protein, we determined the sequence of the N-terminal 25 amino acids from the purified protein (see Materials and Methods). A degenerate oligonucleotide was generated on the basis of a 10-amino-acid sequence and codon usage preferences for *C. crescentus* as described in Materials and Methods.

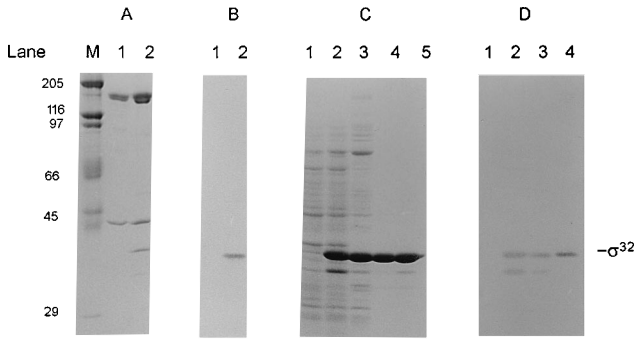


FIG. 1. Purification and identification of *C. crescentus* $\sigma^{32}$ protein. (A) Coomassie blue-stained SDS-polyacrylamide gel of purified *C. crescentus* core enzyme (lane 1) and holoenzyme (lane 2) from RNAP preparations. Lane M, size markers (indicated in kilodaltons). (B) Western blot analysis of RNAP preparations displayed in panel A with an anti-*E. coli* $\sigma^{32}$ antiserum. Lane 1, RNAP core enzyme preparation; lane 2, RNAP holoenzyme preparation. (C) Coomassie blue-stained SDS-polyacrylamide gel of protein preparations during $\sigma^{32}$ purification: lysates of uninduced (lane 1) and IPTG-induced (lane 2) cells, ammonium sulfate precipitation (lane 3), DEAE-cellulose chromatography (lane 4), and heparin-Sepharose chromatography (lane 5). (D) Western blot analysis of purified $\sigma^{32}$ protein with anti-*E. coli* $\sigma^{32}$ antiserum. Lanes 1 and 2 contain the lysates from cells uninduced and induced by IPTG, respectively. Purified *C. crescentus* $\sigma^{32}$ protein and *E. coli* histidine-tagged $\sigma^{32}$ protein are in lanes 3 and 4, respectively.

The end-labeled oligonucleotide was used to probe a cosmid library by colony hybridization. The DNA from one hybridizing clone was isolated and further analyzed by restriction enzyme digestion and Southern hybridization using the same oligonucleotide probe. A 1.8-kb *Eco*RI DNA fragment and a 1.6-kb *Pst*I DNA fragment that hybridized to the labeled probe were subcloned into pBluescript KS to generate plasmids pJW220 and pJW222, respectively (Fig. 2A and B).

To confirm that the cloned fragments contained the *rpoH* gene, the fragments were sequenced on both strands by the dideoxy-chain termination method. DNA sequence analysis showed that the two DNA inserts are overlapping fragments, each of which contains a partial *rpoH* sequence. The 2.2-kb *Pst*I-*Pst*I fragment in the insert of pJW220 was replaced with the 1.6-kb *Pst*I-*Pst*I fragment from pJW222 to yield plasmid pJW250 (Fig. 2C), which now contains the complete *rpoH* gene and flanking sequences. The translated *rpoH* gene encodes a

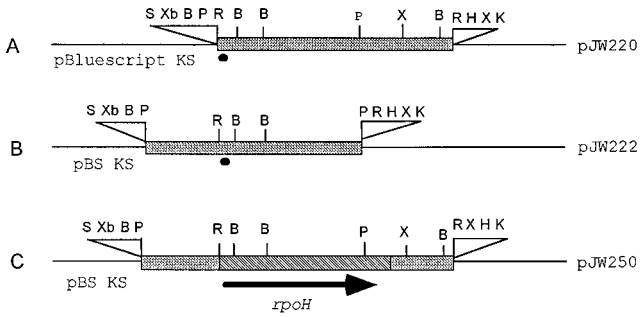


FIG. 2. Location of the open reading frame in cosmid subclones from the *C. crescentus rpoH* gene in plasmid pJW250. The shaded boxes represent *C. crescentus* DNA in plasmid subclones pJW220 (A) and pJW222 (B). The *rpoH* open reading frame in combined clone pJW250 (C) is indicated by the striped box, and the direction of $\sigma^{32}$ translation is indicated by the arrow. The black dot shows the region of hybridization to the degenerated oligonucleotide (OY1; Materials and Methods). Restriction enzyme sites: B, *Bam*HI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; R, *Eco*RI; S, *Sst*I; Xb, *Xba*I; X, *Xho*I.

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Ccres 1      ..MAVNSLSVMSPDGGLSRYLTEIRKFPMLSKDEEFMLAQRWKEHQDPQA
Citfr 1      MTKEMQNL.ALAPVGNLESYIRAANAWPMLSADEERALAEKLHYQGDLEA
Ecoli 1      MTDKMQSL.ALAPVGNLDSYIRAANAWPMLSADEERALAEKLHYHGDLEA
Paeru 1      MTTSLQPVHALVPGANLEAYVHSVNSIPLLSPEQERELAERLFYQQDLEA
                                |----------1.2-----------|  |--

Ccres 49     AHKMVTSHLRLVAKIAMGYRGYGLPIGEVISEGNVGLMQAVKKFEPEKGF
Citfr 50     AKTLILSHLRFVVHVARNYAGYGLRQADLIQEGNIGLMKAVRRFNPEVGV
Ecoli 50     AKTLILSHLRFVVHIARNYAGYGLPQADLIQEGNIGLMKAVRRFNPEVGV
Paeru 51     ARQMVLAHLRFVVHIAKSYSGYGLAQADLIQEGNVGLMKAVKRFNPEMGV
             -------2.1-----------|--------2.2--------|---2.3--
               (DNA melting)         (core binding)      (DNA

                                  |--------Region C--------|
Ccres 99     RLATYAMWWIRASIQEYILRSWSLVKMGTTAAQKKLFFNLRKAKSQIAAF
Citfr 100    RLVSFAVHWIKAEIHEYVLRNWRIVKVATTKAQRKLFFNLRKTKQRLGWF
Ecoli 100    RLVSFAVHWIKAEIHEYVLRNWRIVKVATTKAQRKLFFNLRKTKQRLGWF
Paeru 111    RLVSFAVHWIKAEIHEFILRNWRIVKVATTKAQRKLFFNLRSQKKRLAWL
             --------|-------2.4----------|  RpoH Box  |-------
             melting)  (-10 recognition)

Ccres 149    QEGDLHPDQVSQIATKLGVLDSEVISMNRRLSGPDASLNAPLRADGESEW
Citfr 150    NQ.....DEVEMVARELGVSSKDVREMESRMAAQDMTFDMSSDDESDSQP
Ecoli 150    NQ.....DEVEMVARELGVTSKDVREMESRMAAQDMTFDLSSDDDSDSQP
Paeru 151    NN.....EEVHRVAESLGVEPREVREMESRLTGQDMAFDPAADADDESAY
             ------------------------------3.1---------------|
                          H - T - H
                    (promoter clearance)

Ccres 199    QD..WLADEEQVSQETRVAEDEEKSLRMSLLEEAMVELTDRERHILTERR
Citfr 195    MAPVLYLQDKSSNFADGIEEDNWEDQAANKLTHAMEGLDERSQDIIRARW
Ecoli 195    MAPVLYLQDKSSNFADGIEDDNWEEQAANRLTDAMQGLDERSQDIIRARW
Paeru 196    QSPAHYLEDHRYDPARQLEDADWSDSSSANLHEALEGLDERSRDILQQRW
               |--------3.2--------|          |--------4.1------
                  (core binding)

Ccres 247    LKDD.PTTLEELAAQYGVSRERVRQIEVRAFEKLQKTMREAAIAKNMVDA
Citfr 245    LDEDNKSTLQELADRYGVSAERVRQLEKNAMKKLRAAIEA.........
Ecoli 245    LDEDNKSTLQELADRYGVSAERVRQLEKNAMKKLRAAIEA.........
Paeru 246    LSEE.KATLHDLAEKYNVSAERIRQLEKNAMSKLKGRILA.........
             --|  |----------------4.2-----------|
                          H - T - H
                    (-35 recognition)
```

FIG. 3. Alignment of amino acid sequences of bacterial $\sigma^{32}$ proteins. The predicted product of the *C. crescentus rpoH* gene (Ccres) displays 41% identity to *E. coli* $\sigma^{32}$ (Ecoli), 38% identity to *C. freundii* RpoH (Citfr), and 40% identity to *P. aeruginosa* $\sigma^{32}$ (Paeru). The structural regions found in most $\sigma$ factors are indicated by numbers and have been described previously (22, 28). The conserved nine-amino-acid RpoH box (36) found only in $\sigma^{32}$ proteins is indicated in boldface. The potential helix-turn-helix (H-T-H) DNA binding motifs are underlined.

protein of 295 amino acid residues with a predicted molecular weight of ca. 33,659 (Fig. 3).

**Structural similarity of RpoH homologs.** The deduced product of the *C. crescentus* gene is highly homologous to a large family of bacterial sigma factors, particularly to the heat shock $\sigma^{32}$ subfamily (Fig. 3). The predicted amino acid sequence of this gene displays 41% identity to the *E. coli* $\sigma^{32}$ factor sequence (19, 26, 47), 40% identity to the *P. aeruginosa* $\sigma^{32}$ protein sequence (6, 33), and 38% identity to the *C. freundii* $\sigma^{32}$ protein sequence (17). In addition, *C. crescentus* $\sigma^{32}$ displays 40% sequence identity to the *Myxococcus xanthus* sigma factors SigB (2) and SigC (3) and 42% identity to the *Stigmatella aurantiaca* SigB protein (41) (data not shown; see Discussion).

Comparison of *C. crescentus* $\sigma^{32}$ with other sigma factors, including $\sigma^{32}$ and $\sigma^{70}$, revealed that *C. crescentus* $\sigma^{32}$ contains the typical structural and functional regions found in all sigma factors (Fig. 3) (reviewed in references 22 and 28) except members of the $\sigma^{54}$ factor family (reviewed in reference 31). In addition, the four $\sigma^{32}$ proteins examined in Fig. 3 contain a conserved segment termed the RpoH box which is not present in the other sigma factors (36). The RpoH box, a nine-amino-acid sequence [Q(R/K)KLFFNLR] located between regions 2.4 and 3.1, overlaps the C region, which has been proposed to be important for the DnaK-DnaJ chaperon-mediated negative feedback control of heat shock response (35, 46).

**Functional analysis of *C. crescentus rpoH* gene in vivo.** To determine the function of the *C. crescentus rpoH* gene, we examined whether it could complement *E. coli* KY1608, an *rpoH*

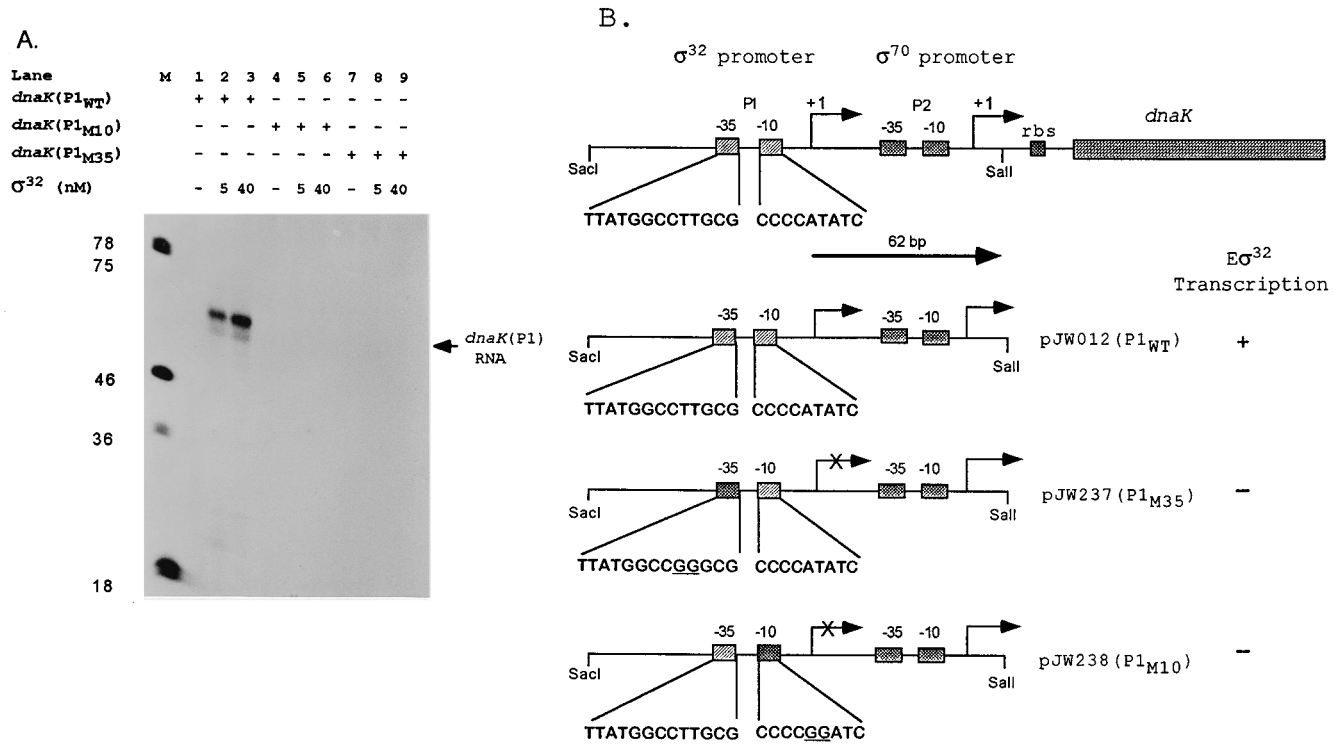


FIG. 4. Effects of promoter mutations on transcription of *dnaK* by *C. crescentus* E$\sigma^{32}$ in vitro. (A) In vitro transcription assays using *C. crescentus* E$\sigma^{32}$ RNAP reconstituted with 1 μM RNAP core enzyme alone or with various concentrations of purified $\sigma^{32}$ as indicated. The *Sac*I-*Sal*I DNA fragment from plasmid pJW012 was used as the template for the wild-type promoter ($P1_{WT}$) (lanes 1 to 3). The *Sac*I-*Sal*I fragments from pJW237 (lanes 4 to 6) and pJW238 (lanes 7 to 9) were used as templates for the two mutant promoters. Sizes are indicated in nucleotides. (B) A schematic diagram of the *C. crescentus dnaK* gene and its upstream promoter region (4) is shown at the top. The *dnaK* gene contains two promoters, a typical heat shock promoter (P1) and a potential housekeeping promoter (P2). Plasmid pJW012 contains the *Sac*I-*Sal*I DNA fragment with both wild-type promoters. Plasmid pJW237 contains a mutant P1 promoter ($P1_{M35}$) in which two highly conserved bases (TT) in the −35 region have been changed to GG. Plasmid pJW238 contains a mutant P1 ($P1_{M10}$) in which two highly conserved bases (AT) in the −10 region have been substituted by GG. The predicted length of the in vitro runoff transcript from promoter P1 is 62 nucleotides and is indicated by an arrow. rbs, ribosome binding site.

null mutant that behaves as a temperature-sensitive mutation and grows only at temperatures below 20°C (48). Cells of strain KY1608 were transformed with either plasmid pJW245, containing the *rpoH* gene under control of the *lac* promoter, or pJW246, containing the *rpoH* gene in the opposite orientation. Equal numbers of colonies were formed on ML agar (200 colonies per plate) by strains containing either of the plasmids at 20°C. However, only the strain carrying plasmid pJW245 with *rpoH* in same orientation as the *lac* promoter formed colonies at 37°C (200 colonies per plate), and no colonies were formed at the high temperature by the strain carrying plasmid pJW246 with *rpoH* in the opposite orientation. These results demonstrated that the *C. crescentus rpoH* gene is capable of complementing the temperature-sensitive defect of the *E. coli rpoH* null mutant strain. Thus, the *C. crescentus* $\sigma^{32}$ protein appears to be a functional and structural homolog of *E. coli* $\sigma^{32}$.

**Overexpression and purification of the *C. crescentus rpoH* gene product.** To analyze the function of *C. crescentus* RpoH protein, we overexpressed *rpoH* in *E. coli* and purified the gene product to near homogeneity. The full-length *C. crescentus rpoH* gene was fused translationally to the T7 promoter in expression vector pRSET(A) (25) to create plasmid pJW255. The size of the overexpressed protein (Fig. 1C, lane 2), which was absent in the uninduced cell lysates (Fig. 1C, lane 1), is close to the predicted size (34 kDa) of the *rpoH* gene product. $\sigma^{32}$ protein at various steps of purification is shown in Fig. 1C (Materials and Methods). A protein band migrating faster than the $\sigma^{32}$ band was observed on SDS–polyacrylamide gels at all steps of the purification (Fig. 1C), and Western blot analysis indicated that it may be a degradation product of the $\sigma^{32}$ protein (Fig. 1D).

The $\sigma^{32}$ protein samples were also monitored by Western blot analysis with an anti-*E. coli* $\sigma^{32}$ antiserum (Fig. 1D). The *E. coli* $\sigma^{32}$ antibody recognized the overproduced protein from IPTG-induced cells (Fig. 1D, lane 2), the purified *C. crescentus* $\sigma^{32}$ (Fig. 1D, lane 3), and the purified histidine-tagged *E. coli* $\sigma^{32}$ (Fig. 1D, lane 4), but it did not react with any protein from the uninduced cell lysates (Fig. 1D, lane 1). The predicted 34-kDa $\sigma^{32}$ of *C. crescentus* and the 32-kDa $\sigma^{32}$ of *E. coli* migrated at approximately the same position (Fig. 1D, lanes 3 and 4), presumably because of the histidine tag on the *E. coli* protein.

**Functional analysis of *C. crescentus* $\sigma^{32}$ in vitro.** We have analyzed the promoter specificity of purified $\sigma^{32}$ in the presence of *C. crescentus* RNAP core enzyme. The activity of the reconstituted holoenzyme was examined in in vitro transcription assays using the *Sac*I-*Sal*I DNA fragment of the *C. crescentus dnaK* gene as the template (18) (Fig. 4B). This fragment bears a typical $\sigma^{32}$ consensus promoter (P1; Table 1), which is transcribed in vivo at a higher level under conditions of heat shock (4). Although a transcript was not observed with RNAP core alone (Fig. 4A, lane 1), a transcript was detected with the addition of 5 nM purified $\sigma^{32}$ (lane 2), and transcription was stimulated by the addition of more purified $\sigma^{32}$ (lane 3). The RNA product migrated at ca. 62 nucleotides, the predicted size

TABLE 1. Comparison of DNA sequences of $\sigma^{32}$-like promoters from *E. coli* and *C. crescentus*

| Promoter | Sequence | | | Reference(s) |
|---|---|---|---|---|
| | −35 region | Spacing (nucleotides) | −10 region | |
| *E. coli* | | | | |
| *dnaK* P1 | TCTCCCCCTTGAT | 14 | CCCCATTTA | 11 |
| *dnaK* P2 | TTGGGCAGTTGAA | 13 | CCCCTATTA | 11 |
| *groE* | TTTCCCCCTTGAA | 13 | CCCCATTTC | 11 |
| *lon* | TCTCGGCGTTGAA | 14 | CCCCATATA | 11 |
| *rpoD* Phs[a] | TGCCACCCTTGAA | 15 | GACGATATA | 11 |
| *C. crescentus* | | | | |
| *dnaK* P1 | TTATGGCCTTGCG | 14 | CCCCATATC | 4, 18 |
| *rpoH* P2 | TACCGCTCTTCAA | 13 | AACTATCTA | This study (Fig. 5B) |
| Consensus | TNNCNCCCTTGAA | 13–15 | CCCCATNTA | |
| Identity[b] | 7 5 55577665 | | 557566 75 | |

[a] Phs, heat shock promoter.
[b] Number of residues at each position identical to the promoter consensus.

of a transcript initiated from promoter P1 of *dnaK* (Fig. 4B), which has been mapped by in vivo primer extension (18). These results suggest that the transcripts are $\sigma^{32}$ specific and $\sigma^{32}$ concentration dependent.

The promoter specificity of the purified $\sigma^{32}$ protein was confirmed by examining two-base substitution mutations in the −35 region (TT to GG) and −10 region (AT to GG) of the $\sigma^{32}$-dependent promoter P1 in plasmids pJW237 and pJW238, respectively (Fig. 4B). As expected, P1-specific transcripts were observed only in the presence of $\sigma^{32}$ protein and a wild-type *dnaK* P1 template, pJW012 (Fig. 4A, lanes 2 and 3). *dnaK* P1-specific transcripts were not detected in the absence of $\sigma^{32}$ protein (lanes 1, 4, and 7) or in the presence of templates containing either *dnaK* P1 promoter mutation, even at higher concentrations of $\sigma^{32}$ protein (lanes 5, 6, 8, and 9). Thus, we conclude that the purified *C. crescentus* $\sigma^{32}$ specifically recognizes the heat shock promoter P1 of the *dnaK* template.

**Identification of the *rpoH* transcription start sites.** The start sites of *rpoH* transcription were determined by the S1 nuclease protection assay (see Materials and Methods). Two protected fragments were observed when 50 μg (Fig. 5A, lane 3) and 100 μg (lane 4) of total mRNA isolated from *C. crescentus* cells grown at 30°C were used in the assays: a larger protected fragment (*rpoH* P1) and a more heavily labeled, smaller fragment (*rpoH* P2). No RNA was protected in the control reaction, which contained only tRNA (lane 2). The start site corresponding to P1 was mapped at a C residue 138 bp upstream of the *Eco*RI site, and the start site corresponding to P2 was mapped at a T residue 70 bp upstream of the same *Eco*RI site (Fig. 5B).

These results indicate that two promoters, *rpoH* P1 and *rpoH* P2, may be involved in the control of *rpoH* gene expression at 30°C. Comparison of sequences 5′ to the two transcription start sites with known promoter consensus sequences showed that the −10, −35 P1 sequence (Fig. 5B) displays some similarity to the unusual *C. crescentus* $\sigma^{70}$ promoter consensus (30) (see Discussion). Surprisingly, the −10, −35 P2 sequence (Fig. 5B) is similar to sequences of the $\sigma^{32}$ promoters (Table 1) identified in both *E. coli* (12) and *C. crescentus* (18). The −10 region of P2 contains 5 of 8 bp identical to the −10 consensus sequence of heat shock promoters, while the −35 region contains 8 of 10 bp identical to the −35 consensus sequence (Table 1). We could not, however, identify sequences upstream of P1 and P2 transcription start sites with obvious similarity to the consensus sequence of *E. coli* $\sigma^{E}$-dependent promoters, which are found in *rpoH* genes of many bacteria. This result was unexpected because $\sigma^{E}$ is known to be an essential component of temperature-induced transcription of the *rpoH* gene in *E. coli* (15, 42).

**Regulation of *rpoH* gene expression during heat shock.** We examined the heat shock regulation of *rpoH* by measuring the expression from the P1 and P2 promoters by S1 nucleases protection assays of RNA isolated from cells grown at either 30 or 42°C (Fig. 6). Levels of the P1 transcript remained relatively constant at both temperatures, while levels of the P2-specific transcripts increased substantially when the temperature was shifted from 30 to 42°C. The levels of P2 RNA also increased as the time at 42°C increased. Quantification by PhosphorImager analysis indicated that the P2/P1 transcript ratio increased from ca. 8 at 30°C to 31 when the temperature was shifted to 42°C for 2 min and to 97 when the cells remained at 42°C for 5 min (Table 2). During the 5-min heat shock, the level of the

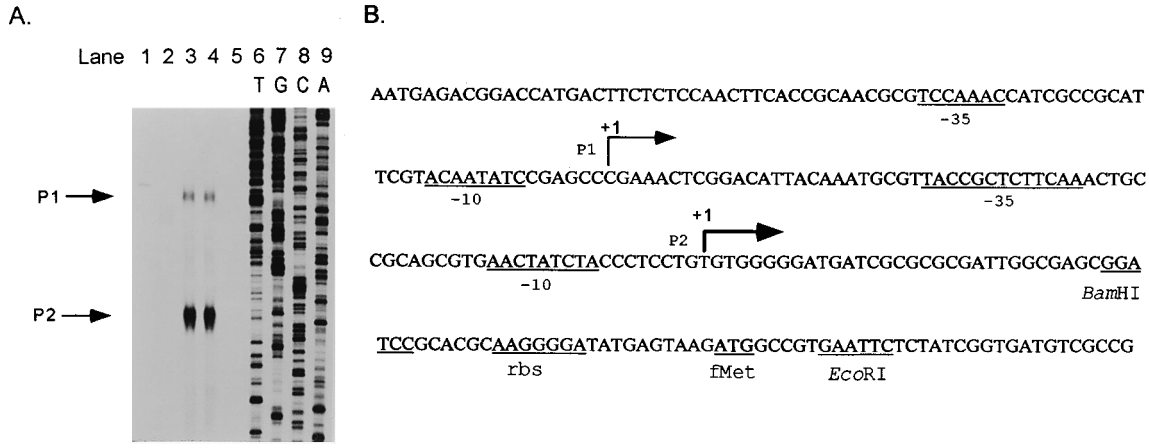


FIG. 5. Mapping of *rpoH* transcription start site. (A) S1 nucleases mapping of the 5′ ends of the *rpoH* transcripts. A single-stranded 0.5-kb *Pst*I DNA fragment uniformly labeled with [α-$^{32}$P]dATP was used as a probe (lane 1) and hybridized (65°C) with 50 μg of tRNA (lane 2) or 50 μg (lane 3) or 100 μg (lane 4) of mRNA isolated from cells of *C. crescentus* wild-type strain CB15. The protected fragments are indicated by arrows. The sequencing ladder (lanes 6 to 9) was generated by using the same oligonucleotide primer which was used to generated the probe. (B) Upstream sequences of the *rpoH* gene and locations of transcription start sites (arrows) and the potential −10 and −35 sequences (underlined) of *rpoH* gene promoters.

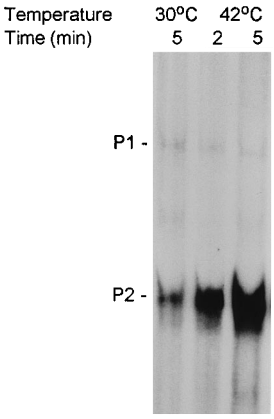


FIG. 6. Effect of heat shock on expression of the *rpoH* P1 and P2 promoters. S1 nuclease protection assays of *rpoH* transcripts were carried out on 50 μg of total RNA isolated from *C. crescentus* cells grown at 30 or 42°C for 2 and 5 min. The reaction conditions and the probe were the same as described in the legend to Fig. 5 and Materials and Methods. P1 and P2 indicate the protected fragments generated by transcripts from the *rpoH* P1 and P2 promoters, respectively.

P1 transcript decreased by ca. 20%, while the level of the P2 transcript increased by approximately 10-fold. Thus, these results suggest that only the P2 promoter, which is most similar to the $\sigma^{32}$ consensus, is heat shock inducible.

## DISCUSSION

**Identification of the *C. crescentus* $\sigma^{32}$ protein and *rpoH* gene.** Many developmental events in *C. crescentus* are regulated by differential gene transcription. Using biochemical, immunological, and genetic approaches, we have identified a protein tightly associated with purified RNAP holoenzyme preparations as the heat shock sigma factor $\sigma^{32}$. We have also isolated the *rpoH* gene encoding the $\sigma^{32}$ protein and shown that it complements the growth defect of a temperature-sensitive *E. coli rpoH* deletion strain at elevated temperatures. Evidence that the purified *rpoH* gene product is the heat shock sigma factor includes its cross-reaction with an *E. coli* anti-$\sigma^{32}$ antibody and specific stimulation of transcription initiation from a *C. crescentus* heat shock promoter.

**In vitro recognition of a *C. crescentus* heat shock promoter.** At least 15 heat shock promoters are known to be transcribed in *E. coli*. These promoters differ from $\sigma^{70}$-dependent promoters with respect to their −35 consensus sequence (TNNCNCC CTTGAA), their −10 consensus sequence (CCCCATNTA), and the length of the spacer (13 to 15 nucleotides) between these two regions (12). In general, they are recognized by E$\sigma^{32}$ both in vivo and in vitro (48). We have shown that the *C. crescentus dnaK* P1 promoter, which is responsible for temperature-induced increase of *dnaK* transcription in vivo (4), is recognized in vitro by a reconstituted transcription system containing purified *C. crescentus* $\sigma^{32}$ protein and core enzyme (Fig. 4A). The specificity of *dnaK* P1 recognition by the E$\sigma^{32}$ was confirmed by demonstrating that mutations either in the −10 or −35 consensus sequence of the P1 promoter abolished transcription initiation. In addition, the same reconstituted *C. crescentus* E$\sigma^{32}$ could not recognize $\sigma^{70}$-dependent promoters from either *E. coli* or *C. crescentus* (44). These results support our conclusion that the *rpoH* gene identified in these studies encodes a *C. crescentus* heat shock sigma factor.

TABLE 2. Quantitative S1 nuclease mapping of *rpoH* mRNA during heat shock response

| Promoter | Relative amt of RNA[a] | | | |
|---|---|---|---|---|
| | 30°C, 5 min | 42°C, 2 min | 42°C, 5 min | 42°C/30°C, 5 min |
| P1 | 1.0 | 0.9 | 0.8 | 0.8 |
| P2 | 8.0 | 28.0 | 78.0 | 9.8 |
| P2/P1 | 8.0 | 31.0 | 97.0 | |

[a] An S1 nuclease protection experiment (Fig. 6) was quantitated by PhosphorImager quantitation. Data are normalized to those for the P1 band at 30°C.

**Comparison of RpoH protein sequences.** Alignment of the *C. crescentus* $\sigma^{32}$ sequence with sequences of three known $\sigma^{32}$ proteins from *E. coli* (19, 26, 47), *C. freundii* (17), and *P. aeruginosa* (6, 33) showed that they contain the four typical functional regions present in sigma factors belonging to the $\sigma^{70}$ class (reviewed in references 22 and 28) and the potential helix-turn-helix motifs in regions 3.1 and 4.2 (Fig. 3). There are, however, two stretches of extra amino acids in the *C. crescentus* protein. One of these stretches consists of five residues within region 3.1, which has been suggested to be involved in promoter clearance in the *E. coli* $\sigma^{70}$ factor (23); the second sequence consists of an additional 10 amino acids at the C terminus (Fig. 3). None of our results shed light on the possible significance of these sequences.

Nakahigashi et al. (36) have now compared the sequences of *rpoH* genes from the five gram-negative bacteria belonging to the γ group (*Enterobacter cloacae*, *Serratia marcescens*, and *Proteus mirabilis*) and α group (*Agrobacterium tumefaciens* and *Zymomonas mobilis*) of bacteria. The most significant amino acid motif to emerge from those studies is the nine-amino-acid residue RpoH box sequence Q(R/K)KLFFNLR located between regions 2.4 and 3.1 in all of these translated proteins as well as in the RpoH sequences of *C. crescentus*, *E. coli*, *C. freundii*, and *P. aeruginosa* (Fig. 3). This RpoH box is not present in other sigma factors, including $\sigma^{70}$ and secondary sigma factors. The RpoH-specific domain is very close to the −10 promoter recognition region (region 2.4) and might reflect the unique promoter specificity of the $\sigma^{32}$ factors. This sequence is also within the so-called C region of $\sigma^{32}$ protein, which has been proposed to function in binding of the DnaK-DnaJ complex and mediating the translation and degradation of *E. coli* $\sigma^{32}$ protein (34, 46). Therefore, it is reasonable to speculate that the RpoH-specific sequences may be involved in the chaperon-mediated negative control of the synthesis and degradation of $\sigma^{32}$ proteins.

The $\sigma^{32}$ proteins are also closely related to the SigB (2) and SigC (3) proteins of *M. xanthus* and to the SigB protein of *S. aurantiaca* (41). The *M. xanthus* SigC protein and the *S. aurantiaca* SigB protein have not been demonstrated to be involved in heat shock response. The SigB protein of *M. xanthus* is essential for late-stage cell differentiation, however, which suggests a developmental role for this protein.

**Identification of *rpoH* promoters.** We have identified two transcription start sites located upstream of the *C. crescentus rpoH* gene. The sequence 5′ to one of them (P1) is similar to the *C. crescentus* $\sigma^{70}$ promoter consensus, which differs from that described for the $\sigma^{70}$-dependent promoters in *E. coli* (30). The −35 region is similar, but not identical, to the corresponding region of the *E. coli* $\sigma^{70}$ consensus, but the −10 region is significantly different from its *E. coli* counterpart. The *rpoH* P1 promoter contains four of the seven consensus residues at −10 and six of the eight consensus residues at −35, which suggests that the *rpoH* P1 promoter may be recognized by $\sigma^{70}$ holoenzyme. The P2 promoter sequence, however, is very similar to the $\sigma^{32}$ promoter consensus (Table 1; Fig. 5B). None of the

*rpoH* genes investigated previously have been reported to contain $\sigma^{32}$-like promoters. We have also shown that the elevated levels of *rpoH* transcription during heat shock response in *C. crescentus* originate primarily from this $\sigma^{32}$-like promoter P2 (Fig. 6; Table 2).

In *E. coli*, at least four promoters are responsible for *rpoH* expression. Three of them are transcribed by $E\sigma^{70}$ ($E\sigma^{D}$) (16, 34), while the other is recognized by $E\sigma^{24}$ ($E\sigma^{E}$) (42). Transcription from the $\sigma^{E}$-dependent promoter in *E. coli* is strongly stimulated at 42°C. At a more extreme temperature (50°C), transcription from the $\sigma^{70}$-dependent promoter shuts down, while transcription from the $\sigma^{E}$-dependent promoter proceeds at a higher level. $\sigma^{E}$-like promoters have also been found upstream of the *rpoH* genes from the $\gamma$ subgroup eubacteria *E. cloacae*, *S. marcescens*, and *P. mirabilis* but not from the $\alpha$ subgroup eubacteria *A. tumefaciens* and *Z. mobilis* (36). The *C. crescentus rpoH* gene, like those of other $\alpha$ subgroup bacteria examined, does not contain a recognizable $\sigma^{E}$-like promoter sequence. The observation that the gene appears to contain a $\sigma^{32}$-dependent promoter sequence suggests that *rpoH* may be positively autoregulated, which would be a unique mechanism for the regulation of heat shock response. We are currently investigating the importance of the $\sigma^{32}$ promoter in the heat shock induction of *rpoH* expression and the role of the heat shock factor RpoH in the developmental regulation of *C. crescentus.*

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## ADDENDUM IN PROOF

The cloning and characterization of the *C. crescentus rpoH* gene has also been reported by Reisenauer et al. (39a).

## REFERENCES

1. **Anderson, D. K., N. Ohta, J. Wu, and A. Newton.** 1995. Regulation of the *Caulobacter crescentus rpoN* gene and function of the purified $\sigma^{54}$ in flagellar gene transcription. Mol. Gen. Genet. **246:**697–706.
2. **Apelian, D., and S. Inouye.** 1990. Development-specific $\sigma$-factor essential for late-stage differentiation of *Myxococcus xanthus*. Genes Dev. **4:**1396–1403.
3. **Apelian, D., and S. Inouye.** 1993. A new putative sigma factor of *Myxococcus xanthus*. J. Bacteriol. **175:**3335–3342.
4. **Avedissian, M., D. Lessing, J. W. Gober, L. Shapiro, and S. L. Gomes.** 1995. Regulation of the *Caulobacter crescentus dnaK* operon. J. Bacteriol. **177:** 3479–3484.
5. **Benson, A. K., G. Ramakrishnan, N. Ohta, J. Feng, A. Ninfa, and A. Newton.** 1994. The *Caulobacter crescentus* FlbD protein acts at *ftr* sequence elements both to activate and repress transcription of cell cycle regulated flagellar genes. Proc. Natl. Acad. Sci. USA **91:**4989–4993.
6. **Benvenisti, L., S. Koby, A. Rutman, H. Giladi, T. Yura, and A. B. Oppenheim.** 1995. Cloning and primary sequence of the *rpoH* gene from *Pseudomonas aeruginosa*. Gene **155:**73–76.
7. **Berk, J. A., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12:** 721–732.
8. **Brun, Y. V., G. Marczynski, and L. Shapiro.** 1994. The expression of asymmetry during *Caulobacter* cell differentiation. Annu. Rev. Biochem. **63:**419–450.
9. **Brun, Y. V., and L. Shapiro.** 1992. A temporally controlled $\sigma$-factor is required for polar morphogenesis and normal cell division in *Caulobacter*. Genes Dev. **6:**2395–2408.
10. **Bukau, B.** 1993. Regulation of the *Escherichia coli* heat-shock response. Mol. Microbiol. **9:**671–680.
11. **Chamberlin, M., R. Kingston, M. Gilman, J. Wiggs, and A. deVera.** 1983. Isolation of bacterial and bacteriophage RNA polymerases and their use in synthesis of RNA *in vitro*. Methods Enzymol. **101:**540–568.
12. **Cowing, D. W., J. C. A. Bardwell, E. A. Craig, C. Woolford, R. W. Hendrix, and C. A. Gross.** 1985. Consensus sequence for *Escherichia coli* heat shock gene promoters. Proc. Natl. Acad. Sci. USA **82:**2679–2683.
13. **Dingwall, A., J. W. Gober, and L. Shapiro.** 1990. Identification of a *Caulobacter* basal body structural gene and a *cis*-acting site required for activation of transcription. J. Bacteriol. **172:**6066–6076.
14. **Ely, B.** 1979. Transfer of drug resistance factors to the dimorphic bacterium *Caulobacter crescentus*. Genetics **91:**371–380.
15. **Erickson, J. W., and C. A. Gross.** 1989. Identification of the $\sigma^{E}$ subunit of *Escherichia coli* RNA polymerase: a second alternate $\sigma$ factor involved in high-temperature gene expression. Genes Dev. **3:**1462–1471.
16. **Fujita, N., T. Normura, and A. Ishihama.** 1987. Promoter selectivity of *Escherichia coli* RNA polymerase: purification and properties of holoenzyme containing the heat-shock $\sigma$ subunit. J. Biol. Chem. **262:**1855–1859.
17. **Garvin, L. D., and S. C. Hardies.** 1989. Nucleotide sequence for the *htpR* gene from *Citrobacter freundii*. Nucleic Acids Res. **17:**4889.
18. **Gomes, S. L., J. W. Gober, and L. Shapiro.** 1990. The expression of the *Caulobacter* heat shock gene *dnaK* is developmentally controlled during growth at normal temperatures. J. Bacteriol. **176:**3051–3059.
19. **Grossman, A. D., J. W. Erickson, and C. A. Gross.** 1984. The *htpR* gene product of *E. coli* is a sigma factor for heat-shock promoters. Cell **38:**383–390.
20. **Hager, D. A., and R. R. Burgess.** 1980. Elution of proteins from sodium dodecyl sulfate-polyacrylamide gels, removal of sodium dodecyl sulfate, and renaturation of enzymatic activity: results with sigma subunit of *Escherichia coli* RNA polymerase, wheat germ DNA topoisomerase, and other enzymes. Anal. Biochem. **109:**76–86.
21. **Haldenwang, W. G.** 1995. The sigma factors of *Bacillus subtilis*. Microbiol. Rev. **59:**1–30.
22. **Helmann, J. D., and M. J. Chamberlin.** 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. **57:**839–872.
23. **Hernandez, V. J., and M. Cashel.** 1995. Changes in conserved region 3 of *Escherichia coli* sigma 70 mediate ppGpp-dependent functions in vivo. J. Mol. Biol. **252:**536–549.
24. **Kornacker, M., and A. Newton.** 1994. Information essential for cell-cycle-dependent secretion of the 591-residue *Caulobacter* hook protein is confined to a 21-amino-acid residue sequence near the *N*-terminus. Mol. Microbiol. **14:**73–85.
25. **Kroll, D. J., H. Abdel-Malek Abdel-Hafiz, T. Marcell, et al.** 1993. A multifunctional prokaryotic protein expression system: overproduction, affinity purification, and selective detection. DNA Cell Biol. **12:**441–453.
26. **Landick, R., V. Vaughn, E. T. Lau, R. A. VanBogelen, J. W. Erickson, and F. C. Neidhardt.** 1984. Nucleotide sequence of the heat shock regulatory gene of *E. coli* suggests its protein product may be a transcription factor. Cell **38:**175–182.
27. **Lane, T., A. Benson, G. B. Hecht, G. J. Burton, and A. Newton.** 1995. Switches and signal transduction networks in the *Caulobacter crescentus* cell cycle, p. 403–417. *In* T. Silhavy and J. Hoch (ed.), Two-component signal transduction. American Society for Microbiology, Washington, D.C.
28. **Lonetto, M., M. Gribskov, and C. A. Gross.** 1992. The $\sigma^{70}$ family: sequence conservation and evolutionary relationships. J. Bacteriol. **174:**3843–3849.
29. **Mager, W. H., and A. J. J. DeKruijff.** 1995. Stress-induced transcriptional activation. Microbiol. Rev. **59:**506–531.
30. **Malakooti, J., S. P. Wang, and B. Ely.** 1995. A consensus promoter sequence for *Caulobacter crescentus* genes involved in biosynthesis and housekeeping function. J. Bacteriol. **177:**4372–4376.
31. **Merrick, M. J.** 1993. In a class of its own-the RNA polymerase sigma factor $\sigma^{54}$ ($\sigma^{N}$). Mol. Microbiol. **10:**903–909.
32. **Mullin, D., S. Minnich, L. S. Chen, and A. Newton.** 1987. A set of positively regulated flagellar gene promoters in *Caulobacter crescentus* with sequence homology to the *nif* gene promoters of *Klebsiella pneumoniae*. J. Mol. Biol. **195:**939–943.
33. **Naczynski, Z. M., C. Mueller, and A. M. Kropinski.** 1995. Cloning the gene for the heat shock response positive regulator (sigma 32 homolog) from *Pseudomonas aeruginosa*. Can. J. Microbiol. **41:**75–87.
34. **Nagai, H., R. Yano, J. W. Erickson, and T. Yura.** 1990. Transcriptional regulation of the heat shock regulatory gene *rpoH* in *Escherichia coli*: involvement of a novel catabolite-sensitive promoter. J. Bacteriol. **172:**2710–2715.
35. **Nagai, H., H. Yuzawa, M. Kanemori, and T. Yura.** 1994. A distinct segment of the $\sigma^{32}$ polypeptide is involved in DnaK-mediated negative control of the heat shock response in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **91:** 10280–10284.
36. **Nakahigashi, K., H. Yanagi, and T. Yura.** 1995. Isolation and sequence analysis of *rpoH* genes encoding $\sigma^{32}$ homologs from gram-negative bacteria: conserved mRNA and protein segments for heat shock regulation. Nucleic Acids Res. **23:**4383–4390.
37. **Neidhardt, F. C., and R. A. VanBogelen.** 1987. Heat shock response in *Escherichia coli* and *Salmonella typhimurium*, p. 1334–1345. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.

38. **Newton, A., and N. Ohta.** 1990. Regulation of the cell division cycle and differentiation in bacteria. Annu. Rev. Microbiol. **44:**689–719.
39. **Ninfa, A. J., D. A. Mullin, G. Ramakrishnan, and A. Newton.** 1989. *Escherichia coli* $\sigma^{54}$ RNA polymerase recognizes *Caulobacter crescentus flbG* and *flaN* flagellar gene promoters in vitro. J. Bacteriol. **171:**383–391.
39a. **Reisenauer, A., C. D. Mohr, and L. Shapiro.** 1996. Regulation of the heat shock $\sigma^{32}$ homolog in *Caulobacter crescentus*. J. Bacteriol. **178:**1919–1927.
40. **Reuter, S. H., and L. Shapiro.** 1987. Asymmetric segregation of heat-shock proteins upon cell division in *Caulobacter crescentus*. J. Mol. Biol. **194:** 653–662.
41. **Skladny, H., M. Heidelbach, and H. Schairer.** 1992. Cloning and DNA sequence of SigB of *Stigmatella aurantiaca*. Nucleic Acids Res. **20:**6416.
42. **Wang, Q., and J. M. Kaguni.** 1989. A novel sigma factor is involved in expression of the *rpoH* gene of *Escherichia coli*. J. Bacteriol. **171:**4248–4253.
43. **Wu, J., A. Benson, and A. Newton.** 1995. FlbD, a global regulator, is required for activation of transcription from $\sigma^{54}$-dependent *fla* gene promoters. J. Bacteriol. **177:**3241–3250.
44. **Wu, J., and A. Newton.** 1994. Unpublished results.
45. **Wu, J., and A. Newton.** 1995. Unpublished results.
46. **Yura, T., H. Nagai, and H. Mori.** 1993. Regulation of the heat-shock response in bacteria. Annu. Rev. Microbiol. **47:**321–350.
47. **Yura, T., T. Tobe, K. Ito, and T. Osawa.** 1984. Heat shock regulatory gene (*htpR*) of *Escherichia coli* is required for growth at high temperature but is dispensable at low temperature. Proc. Natl. Acad. Sci. USA **81:**6803–6807.
48. **Zhou, Y., N. Kusukawa, J. W. Erickson, C. A. Gross, and T. Yura.** 1988. Isolation and characterization of *Escherichia coli* mutants that lack the heat shock sigma factor $\sigma^{32}$. J. Bacteriol. **170:**3640–3649.