

Expression of the *Caulobacter* Heat Shock Gene *dnaK* Is Developmentally Controlled during Growth at Normal Temperatures

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Caulobacter crescentus has a single *dnaK* gene that is highly homologous to the hsp70 family of heat shock genes. Analysis of the cloned and sequenced *dnaK* gene has shown that the deduced amino acid sequence could encode a protein of 67.6 kilodaltons that is 68% identical to the DnaK protein of *Escherichia coli* and 49% identical to the *Drosophila* and human hsp70 protein family. A partial open reading frame 165 base pairs 3' to the end of *dnaK* encodes a peptide of 190 amino acids that is 59% identical to DnaJ of *E. coli*. Northern blot analysis revealed a single 4.0-kilobase mRNA homologous to the cloned fragment. Since the *dnaK* coding region is 1.89 kilobases, *dnaK* and *dnaJ* may be transcribed as a polycistronic message. S1 mapping and primer extension experiments showed that transcription initiated at two sites 5' to the *dnaK* coding sequence. A single start site of transcription was identified during heat shock at 42°C, and the predicted promoter sequence conformed to the consensus heat shock promoters of *E. coli*. At normal growth temperature (30°C), a different start site was identified 3' to the heat shock start site that conformed to the *E. coli* sigma 70 promoter consensus sequence. S1 protection assays and analysis of expression of the *dnaK* gene fused to the *lux* transcription reporter gene showed that expression of *dnaK* is temporally controlled under normal physiological conditions and that transcription occurs just before the initiation of DNA replication. Thus, in both human cells (I. K. L. Milarski and R. I. Morimoto, Proc. Natl. Acad. Sci. USA 83:9517-9521, 1986) and in a simple bacterium, the transcription of a hsp70 gene is temporally controlled as a function of the cell cycle under normal growth conditions.

The heat shock response is an apparently universal phenomenon by which a cell subjected to a sudden increase in temperature induces the synthesis of a small number of highly conserved proteins, the heat shock proteins (hsps) (20, 21, 26). The high degree of conservation of the hsps suggests that these proteins are involved in important processes within the cell.

The best studied among these proteins is the family of polypeptides of M_r 70,000. Eucaryotic organisms typically contain several hsp70-related genes (17, 25, 34). Regulation of these genes is complex; some hsp70 genes are expressed only after cells are stressed, whereas others are expressed constitutively or are developmentally regulated. In contrast, *Escherichia coli* encodes only one hsp70 protein, the product of the *dnaK* gene (4). DnaK is an abundant protein even at normal temperatures (reported to compose 1.4% of the cellular protein), and its synthesis is induced by heat and other forms of stress (15). It is required for the initiation of lambda phage DNA replication (19), is essential for cellular growth, and appears to be required for the initiation of DNA replication at high temperatures (28, 31). We have previously shown that the DnaK homolog from *Caulobacter crescentus* is under complex regulation. In addition to increased synthesis after brief exposure to heat (13, 29) or peroxide (29), under physiologic growth conditions DnaK appears to be synthesized at a defined time in the cell cycle, just before the initiation of DNA replication (13). This response is similar to that observed with the human hsp70 protein, which has been shown in HeLa cells to rapidly increase synthesis during entry into the S phase and to decline in the late-S and G₂ phases (23). Furthermore, the human hsp70 protein is local-

ized to the nucleus in the S phase. At each cell division in *C. crescentus*, only the progeny stalked cell is able to initiate DNA replication (8), and DnaK synthesized in the predivisive cell is selectively localized to this cell upon division (29).

To determine whether a single *dnaK* gene is responsible for both the stress response and the response to temporal control signals, we cloned *dnaK*; it occurred in a single copy on the genome, and we identified both a conserved heat shock promoter and a conserved E σ ⁷⁰ promoter in tandem 5' to the start of the *dnaK* coding sequence. Analysis of *dnaK* transcription in vivo showed that the gene is only transcribed from the heat shock promoter at high temperatures but is transcribed from both promoters in a temporal manner at normal growth temperatures.

MATERIALS AND METHODS

Bacterial strains and plasmids. The synchronizable wide-type strain *C. crescentus* CB15N, used in all experiments, was grown in PYE medium or in modified minimal M2 glucose medium (6). Cultures were synchronized by the method of Evinger and Agabian (12). *E. coli* HB101 was grown in LB medium (24). Plasmid B8, containing the *Drosophila* hsp70 gene cloned into pBR322, was obtained from E. Craig. Plasmid pUCD615, containing the promoterless *luxCDABE* cassette, was obtained from C. I. Kado (16). Plasmid pRK290.20R, a derivative of the broad-host-range plasmid pRK290 (9), contains a multiple restriction site cassette inserted in the *EcoRI* site.

Cloning of the *dnaK* gene. *C. crescentus* genomic DNA was digested with the restriction enzyme *Bam*HI, transferred to nitrocellulose, and hybridized at low stringency (6 \times SSC [1 \times SSC is 0.15 M NaCl plus 0.015 M sodium citrate]-5 \times

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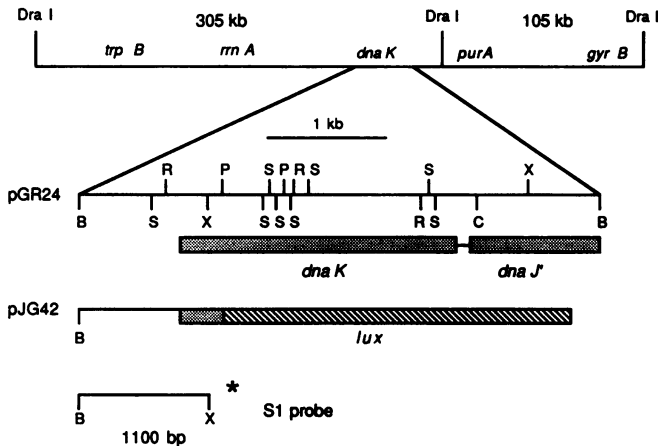


FIG. 1. Map position of the *C. crescentus* *dnaK* gene and a restriction map of a 3.5-kb *Bam*HI fragment containing *dnaK* and a portion of *dnaJ* (pGR24). A subclone, pJG42, containing 968 bp of the *dnaK* 5' region and 370 bp of *dnaK* coding sequence fused to a promoterless *lux* operon is shown below the restriction map. An 1,100-bp *Bam*HI-*Xho*I fragment used for S1 nuclease protection assays is also shown. Restriction enzymes: B, *Bam*HI; C, *Cl*AI; P, *Pst*I; R, *Rsa*I; S, *Sal*I; X, *Xho*I.

Denhardt solution–0.1% sodium dodecyl sulfate at 55°C; washes in 5× SSC at 37°C) to the *Drosophila* hsp70 probe in the B8 plasmid (18). A single band of hybridization in the region corresponding to 3.5 kilobases (kb) was observed. *C. crescentus* genomic DNA was then digested with *Bam*HI and size fractionated by agarose gel electrophoresis. The DNA fragments from 2 to 4 kb in size were then electroeluted and ligated to *Bam*HI-digested pBR322 to create a partial genomic library. After transformation into *E. coli*, the resulting partial library was screened by probing Southern blots of digests of miniplasmid preparations or by colony hybridization with a nick-translated *Drosophila* hsp70 probe in the B8 plasmid. Both methods yielded the same 3.5-kb *Bam*HI fragment.

DNA sequencing. DNA sequence analysis of the entire 3.5-kb cloned fragment was done by the procedure of Sanger et al. (32). Various restriction fragments were subcloned into M13mp18 and M13mp19, and their complete sequences were determined on both strands after deletion subclones were prepared by using the cyclone system from International Biotechnologies. A restriction map of the clone is shown in Fig. 1, and the sequence is shown in Fig. 2.

Nuclease S1 and primer extension mapping. The probe for S1 mapping experiments was a 1.1-kb *Bam*HI-*Xho*I fragment isolated from pGR24 (Fig. 1). S1 nuclease assays were performed as described by Amemiya et al. (1) with the following modifications. Hybridizations were carried out at 90°C for 10 min, followed by transfer to 56°C for 3 h, with 50 µg of *C. crescentus* total RNA. Nuclease S1 digestion was performed with 100 U per sample at 37°C for 30 min. Primer extension mapping (3) was done with a synthetic oligonucleotide (24-mer) that was complementary to nucleotides 433 to 456 of the *dnaK* coding sequence (Fig. 2). The oligonucleotide was obtained from Operon Technologies, Alameda, Calif. The oligonucleotide was 5' end labeled with γ -³²P and hybridized to 50 µg of *C. crescentus* total RNA. Annealing was carried out in 25 µl of 0.1 M piperazine-*N,N'*-bis(2-ethanesulfonic acid) (pH 6.4)–1.0 M NaCl–5 mM EDTA at 45°C for 12 h. The samples were precipitated with ethanol and suspended in 49 µl of 50 mM Tris hydrochloride (pH

8.5)–2 mM dithiothreitol–5 mM MgCl₂–40 mM KCl–0.2 mM deoxynucleoside triphosphates and RNasin (1 U; Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The oligonucleotide was extended at 42°C with 20 U of avian myeloblastosis virus reverse transcriptase (Life Sciences, St. Petersburg, Fla.) for 90 min. The unlabeled oligomer served as a primer for the sequencing ladder, and sequencing was performed by the dideoxynucleotide-chain termination method (32) with [α -³⁵S]ATP.

Construction of *dnaK-lux* transcription fusion. A *dnaK-lux* transcription fusion was made by inserting the 1.35-kb *Bam*HI-*Pst*I fragment from pGR24, containing the entire upstream region plus 370 base pairs (bp) of the coding region of the *dnaK* gene in front of the promoterless *lux* genes in pUCD615 (Fig. 1). The *dnaK-lux* transcription fusion was then subcloned into pRK290.20R, and the resulting recombinant plasmid, pJG42, was mated into *C. crescentus*.

Biochemical techniques. Standard methods were used for DNA end labeling, nick translation, and Northern blots (22). Cellular RNA was purified from *C. crescentus* cells as described by Amemiya et al. (2). Luciferase activity was assayed in *C. crescentus* cultures containing plasmid-borne *lux* genes. To assay the activity of luciferase at defined time points in the cell cycle, synchronized cultures were incubated at 32°C, and then samples were shifted to 25°C for 10 min at the times indicated in Fig. 6B. Because luciferase is irreversibly denatured at temperatures greater than 30°C, this method provides a means of assaying the new synthesis of luciferase over a short period of time. After incubation at 25°C for 10 min, each sample of the culture (optical density at 660 nm, 0.3 to 1.0) was placed in a scintillation vial, and *n*-decylaldehyde (2% emulsion) was added to a final concentration of 0.002%. Emitted light was measured immediately over a period of 6 s in a Packard scintillation counter (model 3330). Counts obtained on the ³H channel were converted into quanta emitted by using a known radioactive standard for calibration (33).

RESULTS

Isolation and sequence analysis of the *dnaK* gene from *C. crescentus*. A single segment of DNA in the *C. crescentus* genome was identified by hybridization of the *Drosophila* hsp70 probe B8 (18) to genomic DNA blots. DNA isolated from the region of the gel that hybridized to the probe was used to construct a *Bam*HI plasmid library in pBR322, as described in Materials and Methods. Two recombinant plasmids that hybridized to the *Drosophila* probe were isolated and shown to have identical restriction maps. The restriction map of the insert in pGR24 is shown in Fig. 1.

The existence of a physical map of the *C. crescentus* genome generated by the ordering of *Dra*I restriction fragments with respect to the genomic map (10) allowed us to determine the general map position of the *dnaK* homolog. We used the cloned fragment as a probe of Southern blots of *Dra*I-digested chromosomal DNA separated by pulsed-field gel electrophoresis (10). The cloned DNA hybridized to a single *Dra*I restriction fragment of 305 kb (Fig. 1). Its relative position was determined by integrating in both orientations a plasmid that contained the *dnaK* gene. This homologous integration introduced another *Dra*I site, and comparison of the restriction fragments generated by insertion in both orientations allowed the placement of *dnaK* near the junction with the adjacent 105-kb fragment (Fig. 1).

To confirm the identity of the cloned fragment as the *dnaK* gene, the fragment was cleaved with various restriction

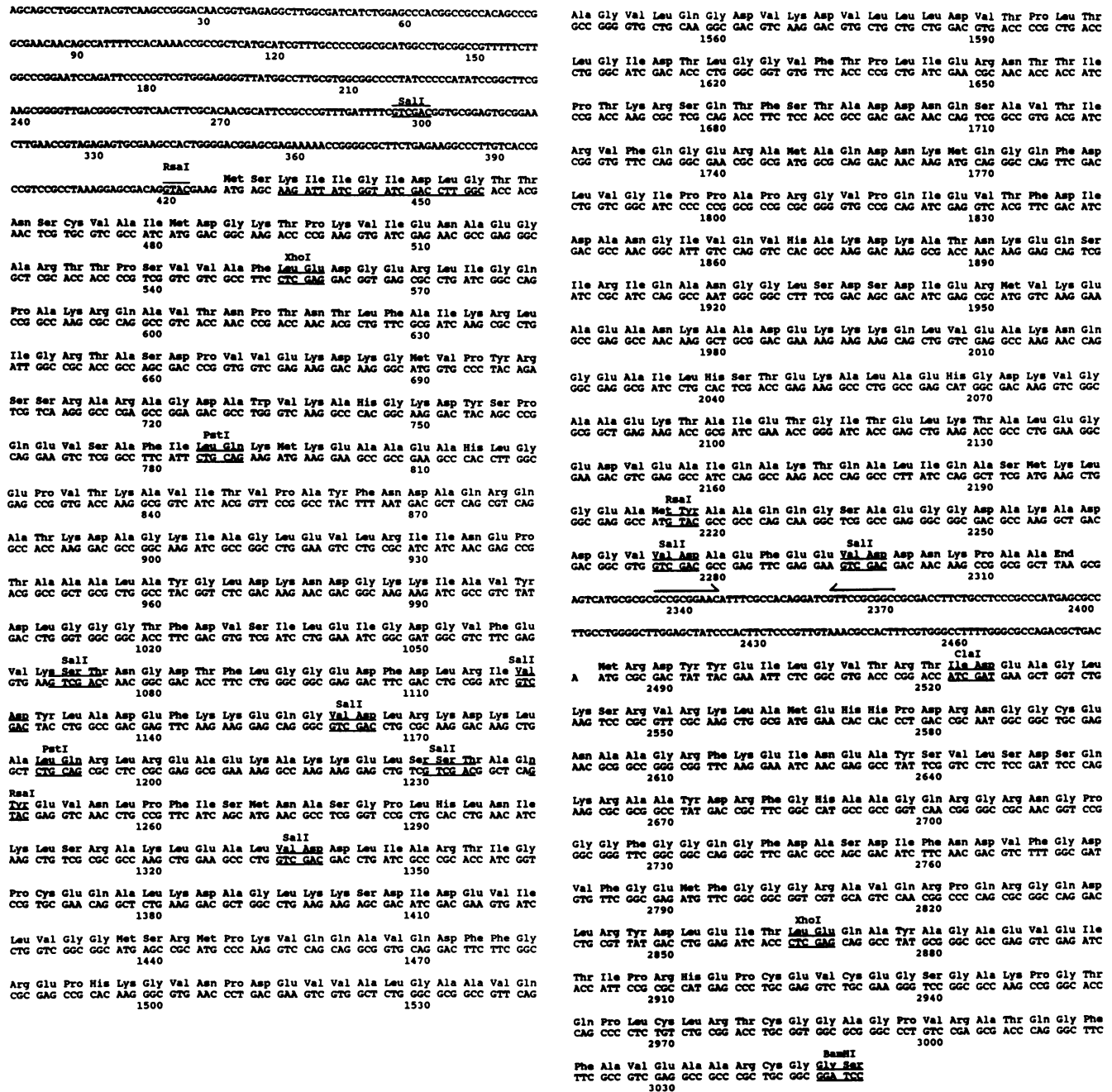


FIG. 2. DNA sequence of the *C. crescentus dnaK* gene and a portion of the adjacent *dnaJ* gene. All of the sequence was determined on both strands and across all restriction sites of the insert in pGR24 shown in Fig. 1. The sequence complementary to the synthetic oligonucleotide (24-mer) is underlined. The inverted repeats in the DNA sequence are indicated by horizontal arrows.

enzymes and subcloned into M13mp18 and M13mp19, and the DNA sequence was determined by the dideoxy-chain termination method (Fig. 2). DNA sequence analysis revealed an open reading frame coding for a protein that was highly homologous to *E. coli* DnaK (Fig. 3). A comparison of the predicted amino acid sequence revealed 68% identity with the *E. coli* DnaK protein (4) and 49% identity with the *Drosophila* hsp70 protein (18). The *dnaK* gene could encode a protein of 67.6 kilodaltons. A second open reading frame

that did not end in the clone was detected downstream of the *dnaK* gene (Fig. 2). The TAA stop codon for the *dnaK* gene was separated from the ATG start codon of the open reading frame by 165 bp. The predicted sequence of the possible 190 amino acids encoded by the open reading frame had 59% identity to the DnaJ protein (5, 27) of *E. coli* (Fig. 3).

Effect of heat shock on the *in vivo* transcription of *dnaK*. The *in vivo* transcript of the *dnaK* gene was detected by Northern blot analysis with total RNA prepared from *C.*

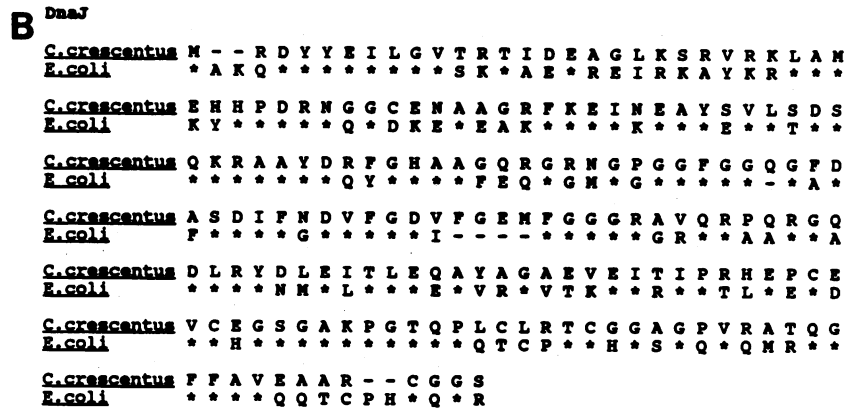


FIG. 3. (A) Comparison of the predicted amino acid sequence of *C. crescentus* DnaK with *E. coli* DnaK (4) and *Drosophila* hsp70 (18). Those amino acids identical in both sequences are indicated with an asterisk. Gaps in the alignment are marked by hyphens within the sequences. (B) Comparison of the predicted amino acid sequence of the open reading frame adjacent to the *dnaK* gene with the amino acid sequence of *E. coli* DnaJ (5, 27).

crescentus either grown at 30°C or heat shocked at 42°C for the times shown in Fig. 4A. The RNA was electrophoretically separated on formaldehyde agarose gels, transferred onto nylon filters, and hybridized to a ³²P-labeled 3.5-kb *Bam*HI fragment from pGR24. The 3.5-kb fragment hybridized to a 4.0-kb mRNA that was present at low levels in cells growing at normal temperatures (30°C) and was transiently induced approximately 10- to 20-fold during heat shock at 42°C, reaching a peak at 15 min. The size of the *dnaK* gene alone is 1.89 kb. The large size of the transcript and its increased expression upon heat shock, together with the DNA sequence data, suggest that the *dnaK* and *dnaJ* genes of *C. crescentus* could be cotranscribed in a manner similar to that found in *E. coli* (30, 35).

The effect of heat shock on the expression of the *dnaK* gene was also determined by S1 nuclease protection assays. A probe was constructed by end labeling the *Bam*HI-*Xho*I fragment shown in Fig. 1. This fragment was hybridized to RNA isolated from cells grown at normal temperatures (30°C) or from cells heat shocked at the times indicated in

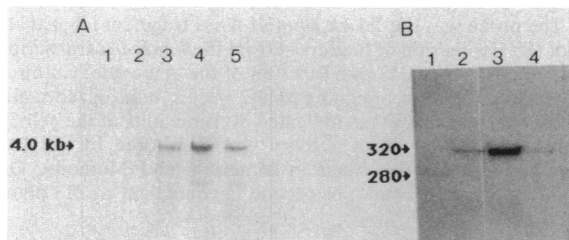


FIG. 4. Effect of heat shock on in vivo transcription of the *dnaK* region. (A) Northern blot analysis of *C. crescentus* RNA with the entire 3.5-kb insert from pGR24 as a probe. Total RNA (5 µg), isolated from cultures grown at 30°C (lane 1) and after 5, 10, 15, and 20 min of exposure to 42°C (lanes 2 through 5, respectively), was subjected to Northern blot analysis as described in Materials and Methods. The major transcript at 4 kb is indicated. (B) Nuclease S1 protection assays of *dnaK* transcripts. The RNA (50 µg) was from cells growing at 30°C (lane 1) and from cultures shifted to 42°C for 10, 15, and 20 min (lanes 2 through 4, respectively). The 5' end-labeled DNA probe was the 1.1-kb *Bam*HI-*Xho*I fragment shown in Fig. 1. The heat shock-protected RNA fragment of 320 bases and the 280-base protected fragment from cells grown at 30°C are indicated.

Fig. 4B. Nuclease S1-resistant fragments were separated on urea-polyacrylamide gels. RNA isolated from cells grown at normal temperatures yielded a 280-nucleotide protected fragment (Fig. 4B), suggesting that the 5' terminus could lie approximately 150 nucleotides upstream of the *dnaK* translation start. A faint fragment of 320 nucleotides was also detected in cells grown at 30°C. Upon heat shock, however, this 320-nucleotide protected fragment transiently increased in amount with time of exposure to 42°C and peaked at 15 min. The heat shock transcript mapped to a region 190 nucleotides upstream of the *dnaK* translation start site.

Identification of the *dnaK* transcription initiation sites by primer extension. A 24-nucleotide synthetic oligomer complementary to nucleotides 433 to 456 of the *dnaK* coding sequence shown in Fig. 2 was end labeled with ³²P and hybridized to RNA isolated either from heat-shocked cells or from cells grown at normal temperature. The hybrids were extended by reverse transcriptase, and the products were resolved in urea-polyacrylamide gels run next to a sequence (ladder). A single extension fragment was obtained with heat-shocked RNA (Fig. 5C, lane 1). The migration of the fragments indicated an initiation site at either the cytidine or guanosine residue at position 236 or 237, respectively (Fig. 5B). Two extension fragments were observed with template RNA from cells grown at normal temperatures (Fig. 5C, lane 2). The less abundant fragment comigrated with the transcript obtained with heat-shocked RNA. The predominant extension fragment corresponded to the smaller transcript detected by S1 protection assays (Fig. 4B). The initiation site of this smaller fragment was at the cytidine or guanosine residue at position 279 or 280, respectively (Fig. 5B).

Sequences 5' to the two transcription start sites were compared with *E. coli* consensus promoter sequences located at positions -10 and -35 (Table 1). Both the sequences at positions -10 and -35 5' to the heat shock transcript (P1) were a close match to the tandem *E. coli* *dnaK* P1 and P2 heat shock promoters. However, the sequences 5' to the start of the transcription of the *C. crescentus* P2 promoter conform, at the -35 region, to the consensus $E\sigma^{70}$ promoter, and the -10 region more closely resembles the $E\sigma^{70}$ -10 region than it does the $E\sigma^{32}$ -10 region.

Temporal control of *dnaK* expression. The time of appearance of the *dnaK* transcript was measured in synchronous

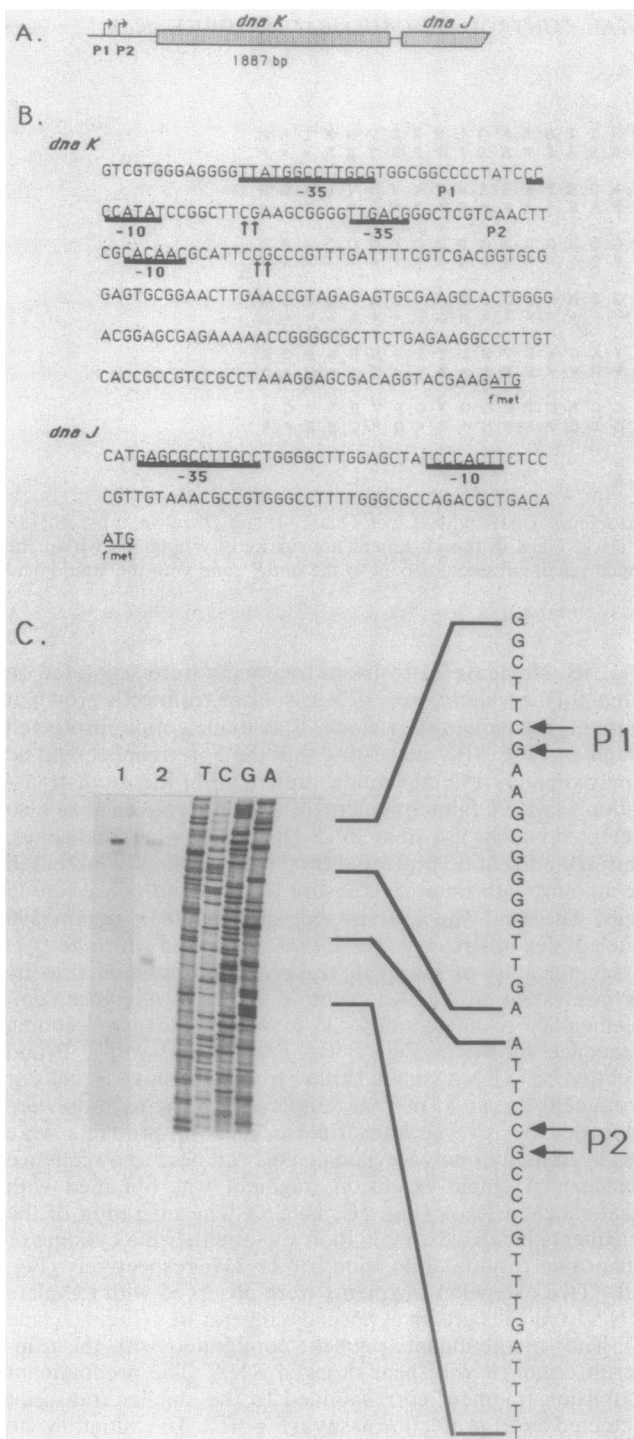


FIG. 5. (A) Schematic diagram of the *dnaK dnaJ* region. (B) Sequence of the 5' region of both the *dnaK* gene and the adjacent *dnaJ* gene. Features of the promoter regions P1 and P2 are indicated. (C) Primer extension mapping of the *dnaK* gene transcriptional start sites. An oligonucleotide complementary to nucleotides 433 to 456 within the *dnaK* coding sequence (underlined in Fig. 2) was synthesized and 5' end labeled with [γ - 32 P]ATP. This probe was hybridized for 12 h at 45°C to 50 μ g of total RNA from heat-shocked cells (lane 1) or from cells grown at 30°C at 0.85 cell cycle division unit (lane 2). Due to the very strong signal obtained with heat-shocked cells, lane 1 was underexposed. The hybrids were then extended by using reverse transcriptase as described in Materials and Methods. The sequencing ladder was generated by using the same 24-mer as a primer and M13mp18 containing the 1.1-kb *Bam*HI-*Xho*I fragment (coding strand). The sequence shown is the complement. The potential transcription start sites are indicated by arrows.

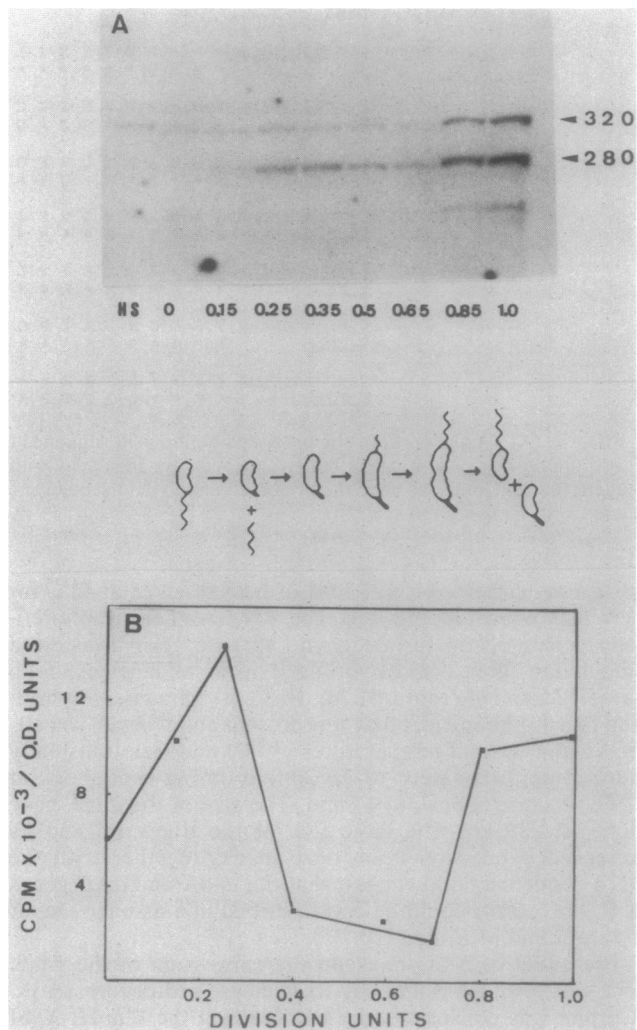


FIG. 6. Expression of the *dnaK* gene as a function of the *C. crescentus* cell cycle. (A) Nuclease S1 protection assays of *dnaK* transcripts isolated from samples of synchronized CB15N cells at the cell division units indicated below the autoradiogram. RNA (50 μ g) was from cells heat shocked for 10 min (HS) or was isolated from cells at 0, 0.15, 0.25, 0.35, 0.5, 0.65, 0.85, or 1.0 cell division unit. The probe was the 1.1-kb *Bam*HI-*Xho*I fragment labeled at the 5' end. (B) Expression of luciferase from the *dnaK-lux* transcription fusion on plasmid pJG42 as a function of the cell cycle. Cultures of *C. crescentus* CB15N containing pJG42 were synchronized, and the synthesis of luciferase at the indicated division units of the cell cycle shown below the graph of activity was monitored by measuring bioluminescence as described in Materials and Methods. O.D., Optical density. One count per second is equivalent to 273 photons emitted per s.

cultures grown at 30°C with the S1 nuclease protection assay (Fig. 6A). Total RNA isolated at different times during the cell cycle was hybridized to a 32 P-labeled 1.1-kb *Bam*HI-*Xho*I probe (Fig. 1) and then treated with S1 nuclease. The 280-nucleotide protected fragment was present at high levels between 0.25 and 0.35 division units, coincident with the initiation of DNA replication. After a period of low transcript levels, the RNA fragment was again detected in large amounts near the end of the cell cycle, just before the initiation of DNA replication in the progeny stalked cell. The 320-nucleotide fragment, which was protected by RNA isolated from heat-shocked cells, was also detected in these

TABLE 1. *E. coli* and *C. crescentus* promoter sequences

Promoter	-35 region	Spacing (bp)	-10 region
$E\sigma^{32}$ consensus	T tC C _o CTTGAA	13-15	CCCCATtT
<i>E. coli</i>			
<i>dnaK</i> P1	TCTCCCCCTTGAT	14	CCCCATTT
<i>dnaK</i> P2	TTGGGCAGTTGAA	13	CCCCTATT
<i>groE</i>	TTTCCCCCTTGAA	13	CCCCATTT
<i>C. crescentus</i>			
<i>dnaK</i> P1	TTATGGCCTTGCG	14	CCCCATAT
<i>dnaJ</i>	GAGCCGCCTTGCC	15	TCCCCTT
$E\sigma^{70}$ consensus	TTGACA	16-18	TATAAT
<i>C. crescentus dnaK</i> P2	TTGACG	15	CACAAC

^a From Cowing et al. (7).

experiments, but at lower levels. At normal physiological temperatures the transcript from the heat shock promoter P1 was temporally controlled, analogous to the transcript from the P2 promoter.

To determine whether the sequences 5' to the start of the *dnaK* gene were sufficient to control its temporal expression, the 1.35-kb *Bam*HI-*Pst*I fragment containing the entire upstream region plus 370 bp of the coding region of the *dnaK* gene (Fig. 1) was subcloned into the transcription reporter vector pUCD615. This vector contains a promoterless *lux* operon from *Vibrio fischeri* (16). The expression of the *lux* genes fused to the *dnaK* promoter was monitored by measuring photon emission as described in Materials and Methods. Expression of the transcription fusion directed by the *dnaK* promoter was cell cycle regulated (Fig. 6B). The promoter region of *dnaK* appeared to initiate transcription of the chimeric gene during the swarmer to stalk transition and again in the predivisional cell just before cell division. In each instance the gene was expressed immediately before the initiation of DNA replication.

DISCUSSION

hsp70 genes are present in a wide variety of species. Genes related to *Drosophila hsp70* have been isolated from organisms as diverse as *E. coli*, *Saccharomyces cerevisiae*, maize, chickens, and humans (21). A high degree of similarity exists among these genes and their encoded proteins. A comparison of the protein-coding region of the *E. coli* and *Drosophila* genes, for instance, reveals 57% identity at the nucleotide level (4). Using a *Drosophila hsp70* gene probe, we isolated a fragment of *C. crescentus* DNA that appeared to encode the DnaK heat shock protein. Sequence analysis revealed that the predicted amino acid sequence of the *C. crescentus* DnaK protein was 68% identical to *E. coli* DnaK and 49% identical to *Drosophila hsp70*. Sequence analysis of the region downstream of the *dnaK* gene revealed a coding sequence for a peptide that was 59% identical to *E. coli* DnaJ.

The heat shock response in *E. coli* is regulated by the *rpoH* gene product, σ^{32} , which directs RNA polymerase to initiate transcription from heat shock promoters at all temperatures (7). The increased synthesis of heat shock proteins after a shift to higher temperatures is caused by increased transcription initiation from heat shock gene promoters by σ^{32} RNA polymerase. Recent experiments have demonstrated that the concentration of $E\sigma^{32}$ transiently increases after a shift to a high temperature, due to an increase in *rpoH*

mRNA level occurring mainly through a posttranscriptional mechanism (11, 14). Three heat-inducible promoters for the operon containing *dnaK* and *dnaJ* genes were identified both in vivo and in vitro in *E. coli*. These promoters are also used at normal temperatures. In *C. crescentus*, the *dnaK* gene also had two transcription start sites, but only one was heat inducible.

The promoter corresponding to the transcript that increased in abundance during the heat shock response, P1, had a -10 region that conforms to the consensus -10 region for heat shock promoters of *E. coli* (4), and its -35 region fits reasonably well with the consensus -35 region of *E. coli* heat shock promoters (Table 1). These results suggest that *C. crescentus* contains a minor sigma factor similar to σ^{32} of *E. coli*. A heat shock protein of 37 kilodaltons was previously detected in *C. crescentus* by immunoprecipitation with anti-RNA polymerase antibody (29). This protein is a candidate for the *C. crescentus* heat shock sigma factor but awaits biochemical analyses with the purified protein.

The P2 promoter, which appears to conform to the $E\sigma^{70}$ promoter and the σ^{70} promoters found in front of some *C. crescentus* genes, was active only during growth at physiologic temperature (30°C) but was under temporal control. All of the regulatory sequences required for temporal control of *dnaK* transcription appeared to be within the 820 bp 5' to the start of transcription. This conclusion is supported by the observation that this 5' regulatory region and 370 bp of *dnaK* coding sequence fused to a promoterless *lux* operon resulted in the same temporal expression of the *lux* activity as the expression of the chromosomal *dnaK* gene. The *C. crescentus dnaK* gene was transcribed just preceding the S phase during the transition from swarmer to stalked cells and again in late predivisional cells just before the initiation of DNA replication in the progeny stalked cell. These results confirm previous observations that the *C. crescentus dnaK* protein is synthesized at defined times in the cell cycle (13).

Transcription at the putative heat shock promoter under normal physiological conditions also varied as a function of the cell cycle, showing an increase at the transition from swarmer to stalked cells and again when cells were beginning to divide (0.85 to 1.0 division units). There are at least two possible explanations for this. In other systems the increase in transcription from heat shock promoters is mediated through increases in the level of σ^{32} . It may be that the comparable heat shock sigma factor gene is under cell cycle modulation and that other heat shock proteins are also increased at this point during the cell cycle. More likely, *cis*-acting sequences that contribute to temporally controlled

modulation of gene expression affect the rate of *dnaK* transcription by both the constitutive but low levels of the heat shock sigma factor and the sigma 70 factor.

The function of DnaK in *C. crescentus* is not known, although there are a number of possible roles for this heat shock protein during the normal cell cycle. DnaK and other heat shock proteins might participate in global control of development in *C. crescentus*. This function may be mediated through stability and proteolysis of cell-type-specific proteins. Alternatively, the DnaK protein might be required for some aspect of *C. crescentus* DNA replication. It is known that the *E. coli* DnaK and DnaJ proteins are required for successful initiation of phage lambda DNA replication (19), and recently a mutant allele of *dnaK* has been shown to cause conditionally defective initiation of *E. coli* DNA replication (31). Maximal transcription of *dnaK* in *C. crescentus* reflects the time of initiation of new rounds of DNA replication; in fact, the DnaK protein specifically segregates to the progeny stalked cell, in which a new round of DNA replication occurs (29). These results are analogous to that seen with the cell-cycle-controlled transcription of the human hsp70 gene in HeLa cells (23). Thus, the expression of a highly conserved heat shock protein in such diverse cell types as a bacterial cell and a human cell shows parallel cell cycle control under normal physiological conditions.

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