DNA protein regulates transcription of the rpoH gene of Escherichia coli

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The rpoH (htpR) gene of Escherichia coli encodes a σ factor which confers upon RNA polymerase the ability to recognize the promoters for genes responsive to the phenomenon termed the heat shock response. dnaA protein, a sequence-specific DNA binding protein, is required for initiation of chromosomal replication by binding to sites within the chromosomal origin. dnaA protein also autoregulates its expression by binding to a site in the dnaA promoter region. Two copies of the dnaA protein recognition sequence are present within the rpoH promoter region. Using filter binding assays, dnaA protein was observed to bind specifically to DNA fragments containing the rpoH promoter region with greater affinity than its binding to the dnaA promoter region. By contrast, reduced binding to a DNA fragment containing the lacUV5 promoter was observed. DNase I footprint analysis indicated that dnaA protein protects specific sites within the rpoH promoter region. The binding of dnaA protein to the rpoH promoter region resulted in transcriptional repression from two of the three promoters of the rpoH gene in vitro. Elevated levels of dnaA protein repressed transcription from these two rpoH promoters in vivo. These results indicate that dnaA protein regulates rpoH transcription to influence the expression of genes under rpoH control.

DNA protein is required for initiation of DNA replication from the Escherichia coli chromosomal origin, oriC, in vivo (Hirota et al., 1970; Zyskind et al., 1977; Kung and Glaser, 1978) and in vitro (Fuller and Kornberg, 1983; Bramhill and Kornberg, 1988). In this process, dnaA protein recognizes and binds cooperatively to sites within the oriC region (Chakraborty et al., 1982; Fuller et al., 1984). Upon binding, this protein appears to induce a localized unwinding of oriC in an ATP-dependent fashion to create a structure required for subsequent steps of replication (Baker et al., 1986; Sekimizu et al., 1987; Bramhill and Kornberg, 1988). These and other results suggest that dnaA protein functions at an early stage in the initiation process.

dnaA protein not only binds to oriC but to other DNA fragments including those containing the dnaA promoter, the mioC promoter, and to origin sequences of some plasmids (Fuller et al., 1984). DNA fragments specifically bound by dnaA protein share a nine base pair (bp) consensus sequence, TTATCAATT, which is postulated to be recognized by dnaA protein. Other experiments indicate that dnaA protein represses transcription of the dnaA and mioC genes (Braun et al., 1985; Atlung et al., 1985; Kücherer et al., 1986; Lother et al., 1985; Stuitje et al., 1986; Wang and Kaguni, 1987). This repressive effect was observed at levels of dnaA protein required for specific binding to these promoter-containing DNA fragments (Wang and Kaguni, 1987). The ability of dnaA protein to autoregulate its expression and its role in initiation of E. coli chromosomal replication may contribute to regulating the frequency of initiation. The binding of dnaA protein to plasmid origins has also been correlated with the involvement of dnaA protein in plasmid DNA replication (Hasunuma and Sekiguchi, 1977; Frey et al., 1979; Ortega et al., 1986; Kline et al., 1986; Hansen et al., 1986; Murakami et al., 1987; Seufert and Messer, 1987). These observations suggest a biochemical function in site-specific binding by dnaA protein.

E. coli exposed to high temperature is induced to express 17 specific proteins in a phenomenon termed the heat shock response (reviewed by Neidhardt and VanBogelen, 1987). Other treatments which elicit this response include ethanol, bacteriophage infection, or UV irradiation. A σ factor, σ27, encoded by the rpoH (htpR) gene confers upon RNA polymerase the ability to recognize and transcribe from the promoters of these heat shock genes (Landick et al., 1984; Grossman et al., 1984; Cowing et al., 1985; Bloom et al., 1986).

Recent reports indicate that the level of σ27 is relatively low at 30 °C and increases by a temperature shift to 42 °C (Lesley et al., 1987; Straus et al., 1987). The increase in σ27 appears to occur by increased transcription and by stabilization of rpoH mRNA (Tilly et al., 1986; Erickson et al., 1987). rpoH transcripts start at promoters designated here as rpoH1P, 2P, 3P, and 4P (Erickson et al., 1987; Tobe et al., 1987). Promoters 1P and 4P are recognized by σ27-RNA polymerase and account for 90% of the total rpoH mRNA at 30 °C (Erickson et al., 1987). rpoH3P may be recognized by a novel form of RNA polymerase. A fourth promoter, 2P, appears to be strain-dependent. A temperature increase results in an increase in rpoH transcript levels with 2P and 3P increasing the most. Despite the response of rpoH expression to increased temperature, its expression does not appear to involve σ27 (Bloom et al., 1986; Erickson et al., 1987).

Computer analysis revealed two presumptive dnaA protein recognition sequences (dnaA boxes) in the promoter region of the rpoH gene. This observation and the property of abnormal cell division in conditionally defective rpoH mutants (Tsuchido et al., 1986) led us to consider whether dnaA protein might regulate expression of the rpoH gene to coordinate initiation

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This paper is dedicated to Arthur Kornberg on the occasion of his 70th birthday.

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1 The abbreviations used are: bp, base pair; PIPES, piperazine-N-N'-bis(2-ethanesulfonic acid); kb, kilobase pair.
of DNA replication to cell division. In this study, we report that dnaA protein binds specifically to the rpoH promoter region as determined by nitrocellulose filter binding assays and DNase I protection experiments. dnaA protein repressed transcription from two promoters of the rpoH gene in vivo and in vitro.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains and Plasmids** — E. coli W3110 and AB1157 thr-1, ara 14, leuB6, Δglt-pncA62, lacY1, trp-5, supE44, thi-1, F- thi-1 were from the E. coli Genetic Stock Center. Plasmid pNG1 (Johnston et al., 1985) was from Dr. Dan S. Ray, UCLA. pDS596 contains the dnaA gene inserted in the vector pNG1 and under control of the araB promoter (Hwang and Kaguni, 1988). The plasmid pdnaA/dnaA contains the dnaA and adjacent tnaA genes within 0.94 and the 3.4-kb EcoRI fragments inserted in the vector pMB45 (Burgers et al., 1981). pFN82, from Dr. Frederick C. Neidhardt, University of Michigan, consists of a 2.5-kb PsiI fragment containing the N-terminal coding region of the rpoH gene inserted at the single PstI site of pBR322 (Neidhardt et al., 1983). pBR322 and pUC19 were from this laboratory. Plasmid DNAs were purified from cleared lysates by centrifugation in ethidium bromide-CsCl gradients. Individual restriction fragments separated by electrophoresis were purified by gel electroelution with an ISCO 1750 electrophoretic purification system. DNA concentrations were determined spectrophotometrically or by comparison of the purified DNA fragments to different known amounts of electrophoretically separated restriction enzyme digests visualized by ethidium bromide staining. The 203-bp EcoRI fragment containing the lacUV5 promoter was a gift from Dr. L. Dorimer (this department (Lorimer and Revin, 1986).

**Enzymes**—Restriction enzymes HindIII, EcoRI, CiaI, EcoRV, and PoulI, DNA polymerase I (large fragment), and T4 polynucleotide kinase were purchased from New England Biolabs; PstI was from Bethesda Research Laboratories; HpaII and S1 nuclease from Pharmacia LKB Biotechnology Inc.; DNase I from Worthington. RNA polymerase holoenzyme was purified from W3110 as described except that Bio-Gel A5m (Bio-Rad) chromatography was replaced by chromatography on a TSK 3000SW (Altax) high performance gel permeation column (Burgers and Jendrisak, 1975; Gonzalez et al., 1977). dnaA protein was purified from an over-producing strain (Hwang and Kaguni, 1988). Protein concentrations were determined by the dyes-binding method with bovine serum albumin as a standard (Bradford, 1976).

**Radioactive Labeling of DNA**—Restriction enzyme digests or purified DNA fragments were either 5'-end labeled with T4 polynucleotide kinase and [α-32P]ATP according to the manufacturer’s instructions or 3'-end labeled with DNA polymerase I (large fragment) and [α-32P]dCTP as described (Wang and Kaguni, 1987).

**DNA Binding Assays**—Nitrocellulose filter binding assays were performed as described (Wang and Kaguni, 1987). DNase I protection assays (Galas and Schmitz, 1978) (10 μl) contained the indicated amounts of dnaA protein and end-labeled DNA fragments in buffer containing 40 mM Tris-HCl, pH 7.6, 100 mM NaCl, 10 mM MgCl2, 0.1 mM EDTA, 2 mM dithiothreitol, 100 μg/ml bovine serum albumin, and 10% glycerol. The PoulI-CiaI fragment labeled at the 5' end as described above was cleaved with HpaII and used without further purification in these assays. Cleavage with HpaII produces a 27-bp PoulI-HpaII fragment and a 156-bp HpaII-CiaI fragment. For the other strand, the PoulI-CiaI fragment was 3' end-labeled at the CiaI site. Reactions were incubated at 37°C for 10 min. DNase I (4 ng) was added and the incubations were continued for 2 min. After addition of an equal volume of stop buffer containing 0.1% sodium dodecyl sulfate, 0.3 M sodium acetate, 25 mM EDTA, and 100 μg/ml tRNA, the samples were ethanol-purified, resuspended in 5 μl of gel loading buffer containing 8% formamide, 10 mM NaOH, 1 mM EDTA, and 0.025% bromophenol blue, boiled for 2 min, and then placed on ice. Electrophoresis was in an 8% sequencing gel.

Maxam-Gilbert (Maxam and Gilbert, 1980) sequencing reactions of the end-labeled DNA served as markers for DNase I protection experiments.

**Run-off Transcription Assays**—In vivo transcription assays were performed as described with the following modifications (Wang and Kaguni, 1987). A prior incubation was performed at 37°C for 10 min in buffer containing 0.025 pmol of the indicated DNA fragment, 0.65 pmol of RNA polymerase, and the indicated amounts of dnaA protein in the absence of ribonucleotides. Heparin (100 μg/ml), 300 μM each of CTP, GTP, ATP, and 50 μM [α-32P]UTP were added to inhibit open complex formation and to initiate transcription. Incubation was continued at 37°C for 10 min. Reactions were stopped, and samples were ethanol-purified, resuspended, and electrophoresed in 8% polyacrylamide gels containing 7 M urea.

Gels were dried onto Whatman DE31 paper and autoradiographed with Kodak XAR-5 film either at room temperature or at −70°C with Cronx Quanta III intensifying screens. The autoradiograms were quantitated by densitometry with a Hoefer gel scanner interfaced with an IBM personal computer.

**Preparation of DNA**—DNA was prepared (Brosius et al., 1982) with the following modifications. E. coli AB1157 containing either pNG1 or pDS596 was grown in Luria broth media (120 ml) (Miller, 1972) at 30°C in a shaking water bath to a turbidity of 595 nm of 0.1. Arabinose was then added to 0.75% (w/v) to induce synthesis of dnaA protein. Incubation was continued at 30°C with shaking, and portions were removed prior to or at the indicated times after arabinose addition. Where indicated, cultures were shifted to 42°C after induced expression of dnaA protein for 2 h at 30°C, and portions were removed at the indicated times. Portions of the culture (3 ml) from each time point were immediately centrifuged in a Fisher microcentrifuge and 20 μl of supernatant was analyzed as described (Brosius et al., 1982). The lysate was phenol-extracted three times, ether-extracted, and total cellular RNA was obtained by ethanol precipitation. RNA concentration was determined spectrophotometrically (1 absorbance unit at 260 nm equals 40 μg/ml).

**S1 Nuclease Assays**—Quantitative S1 nuclease assays (20 μl) were performed in buffer containing 30% PIPES, pH 6.4, 1 mM EDTA, 0.4 M NaCl, 25 μg of yeast tRNA (Sigma), and 80% formamide by hybridization at 45°C for 12-16 h with 25 μg of the isolated RNA to 0.025 pmol of 5' end-labeled EcoRV restriction fragment containing the rpoH promoter region. At the end of hybridization, 300 μl of cold S1 nuclease buffer containing 50 mM sodium acetate, pH 4.6, 0.25 M NaCl, 4.5 mM ZnSO4, 20 μg/ml heat-denatured salmon sperm DNA, and 50 units of S1 nuclease was added. Reactions were incubated at 37°C for 15 min. Ammonium acetate and EDTA were added to 0.25 M and 5 mM, respectively. The samples were precipitated by addition of 2.5 volumes of ethanol and resuspended in 20 μl of gel loading buffer described above. Electrophoresis was in an 8% polyacrylamide gel containing 7 M urea. Autoradiography and its quantitation were as described above.

**RESULTS**

dnaA Protein Binds Specifically to Restriction Fragments Containing the rpoH Promoter Region—Two presumptive dnaA protein recognition sequences (dnaA boxes) were observed in the promoter region of the rpoH gene (Fig. 1, A and B). To examine the binding affinity of dnaA protein to the rpoH promoter region, nitrocellulose filter binding assays were performed. Plasmid pFN82 contains the promoter region and the N-terminal rpoH coding sequence in a 2.5-kb PsiI fragment insert at the PstI site of pBR322 (Neidhardt et al., 1983). HpaII digestion of pFN82 results in fragments including those containing the rpoH promoter region (235 bp) and the pBR322 origin (527 bp). The latter DNA fragment contains a dnaA box near the pBR322 origin, dnaA protein binds to this fragment with affinity similar to its binding to the dnaA promoter region (Fuller et al., 1984; data not shown). Filter binding assays were performed with increasing amounts of dnaA protein (Fig. 2). A DNA fragment near the size expected for the rpoH promoter-containing fragment (235 bp) was bound with higher affinity by dnaA protein than its binding to the 527-bp pBR322 origin fragment (Fig. 2).

Based on DNA sequence information, HindIII digestion of the fragment containing the rpoH promoter (233 bp) is expected to produce two fragments of 186 and 43 bp. A second HindIII site is located in the 622-bp HpaII fragment of the vector. It is not bound by dnaA protein under these reaction conditions. DNA fragments bound with 100 ng of dnaA protein and retained on a nitrocellulose filter were isolated and
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**Fig. 1.** A, physical map of the rpoH promoter region. Pertinent restriction enzyme sites: C, ClaI; H, HindIII; Hp, HpaII; Ru, EcoRV; Ps, PstI; Pu, PvuII. The approximate positions of dnaA boxes (ddf), rpoH promoters (dp), and the rpoH coding region (d) are indicated. Restriction fragments used in the experiments are also indicated. B, DNA sequence of the rpoH promoter region (Landick et al., 1984; Yura et al., 1984; Gill et al., 1986) (coding strand) includes the C-terminal coding region of ftsX, positions of transcriptional start sites for rpoH mRNA (Erickson et al., 1987), dnaA protein recognition sequences (d), the N-terminal coding region of rpoH, and the indicated restriction enzyme sites.

**Fig. 2.** Preferential binding of dnaA protein to a restriction fragment containing the rpoH promoter region. Filter binding assays were performed as described under “Experimental Procedures” with the indicated amounts of dnaA protein and 100 ng of 3' end-labeled pFN82 digested with HpaII used as a size standard; TD, total digest of pFN82; B, bound fragments eluted from the filter; F, fragments which flowed through the filter. HindIII, DNA fragments retained on the filter by 100 ng of dnaA protein were isolated and digested with HindIII prior to electrophoresis.

digested with HindIII restriction enzyme (Fig. 2). The 527-bp fragment containing the pBR322 origin and lacking a HindIII site was not digested. Absence of the fragment near 235 bp and production of subfragments of about 190–195 bp and 40 bp by HindIII confirm that the HpaII fragment bound with greatest affinity by dnaA protein contains the rpoH promoter region.

The relative binding affinity of dnaA protein to fragments containing the promoter regions of dnaA (945 bp), rpoH (659 bp), and lacUV5 (203 bp) was measured in filter binding assays (Fig. 3). With equimolar amounts (in fragment) of each, higher affinity binding by dnaA protein to the rpoH promoter-containing fragment (659 bp) was observed compared to the dnaA promoter-containing fragment (945 bp). The lacUV5 fragment (203 bp) was poorly bound. The increased binding affinity to the rpoH promoter-containing fragment may be due to the presence of two dnaA protein recognition sequences in the rpoH promoter region compared to one such sequence in the region of the dnaA promoters. Alternatively, nucleotides flanking the recognition sequences may influence binding affinity.

dnaA Protein Binds to the dnaA Boxes in the rpoH Promoter—In order to locate the sites of binding by dnaA protein in the rpoH promoter region more precisely, DNase I protection assays (Galas and Schmitz, 1978) were performed. Restriction fragments containing the rpoH promoter region were}

**Fig. 3.** Preferential binding of dnaA protein to restriction fragments containing the dnaA promoter region or the rpoH promoter region. A, filter binding assays were performed as described under “Experimental Procedures” with 0.025 pmol each of 5' end-labeled DNA fragments containing the promoter regions of dnaA, rpoH, and lacUV5. The amounts of dnaA protein added are indicated. TD, total DNA used in the binding assay; B, bound fragments eluted from the filter; F, fragments which flowed through the filter. B, the fraction (in percent) of a DNA fragment retained and eluted from filters is expressed as a ratio to the total amount of that fragment in both the “bound” and “flow-through” lanes measured as described under “Experimental Procedures.”

5' or 3' end-labeled at the ClaI site and incubated with varying amounts of dnaA protein. A limited digestion with DNase I was performed, and the denatured products were separated on a sequencing gel (Fig. 4, A and B). Four regions of protection were observed on either strand in the vicinity of the two 9-base pair dnaA boxes. This pattern of protection was observed at levels of dnaA protein sufficient for specific recognition of rpoH promoter-containing fragments on nitrocellulose filters.

dnaA Protein Inhibits Transcription from Two of the Three rpoH Promoters in Vitro—Transcription of the rpoH gene apparently involves as many as four promoters (Erickson et al., 1987; Tobe et al., 1987). rpoH1P and 4P are recognized by e30-RNA polymerase. A third promoter, 3P, is apparently recognized by a novel form of RNA polymerase which may be distinct from e30 or e30-RNA polymerase. Detection of the fourth promoter, 2P, by S1 mapping was dependent on the E. coli strain from which the RNA was isolated (Erickson et al., 1987).

Different restriction fragments were used in run-off transcription assays to define the start sites and strandedness of the transcripts and to determine the influence of dnaA protein on rpoH transcription. Run-off transcription assays from the rpoH promoters were performed with various preparations of e30-RNA polymerase purified as described (Burgess and Jen-drisk, 1975; Gonzalez et al., 1977). Most preparations resulted in transcription from 1P and 4P (data not shown). This is consistent with results of others that e30-RNA polym-
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FIG. 4. dnaA protein binds to both dnaA boxes in the rpoH promoter region. DNase I protection assays were performed with the indicated amounts of dnaA protein and 3’ end-labeled PvuII-ClaI fragment (A) or 5’ end-labeled HpaII-ClaI region (B) as described under “Experimental Procedures.” The protected regions (c), dnaA protein recognition sequences, and lanes containing the products of Maxam-Gilbert sequencing reactions of the end-labeled DNA fragments used in the DNase I protection assays are also indicated.

FIG. 5. Run-off transcription assays with DNA fragments containing the rpoH promoters. Assays were performed as described under “Experimental Procedures” with the indicated DNA fragments as templates. The positions of transcripts from the rpoH promoters are indicated. M, 3’ end-labeled pBR322 DNA digested with HpaII was included as a size standard.

erase mediates transcription from these promoters. One preparation resulted additionally in transcription from 3P presumably due to the presence of a novel σ factor or a positive regulatory protein. Due to the proximity of the dnaA protein recognition sequences to 3P, this preparation of RNA polymerase was used in the following experiments.

The DNA templates used included the PvuII-ClaI, PstI-ClaI, and EcoRV fragments containing the rpoH promoter region (Fig. 1). Transcription of the 659-bp PvuII-ClaI fragment resulted in three transcripts of about 220, 75, and 65 nucleotides relative to single-stranded DNA marker fragments electrophoresed in a separate lane (Fig. 5). The PstI-ClaI fragment which extends 1.2 kb upstream from the PvuII site was used as a template. Three transcripts were observed with this template and were of similar sizes as those with the PvuII-ClaI fragment. Compared to the PvuII-ClaI fragment, the 342-bp EcoRV fragment is truncated by four nucleotides on the template strand near the N-terminal rpoH coding region (Fig. 1B) and is about 320 bp shorter at the other end. Three transcripts observed with the EcoRV fragment were each slightly shorter than those observed with the PvuII-ClaI fragment. ClaI restriction within the rpoH promoter region of the template DNA strand produces a 5’ end 16 nucleotides from the first codon. Taking this distance into account, these experiments indicate transcriptional start sites at approximately 235, 90, and 80 nucleotides upstream from the coding sequence. Whereas these transcripts have not been mapped relative to the products of sequencing reactions of the template fragment, these results are consistent with results of others (Erickson et al., 1987; Tobe et al., 1987) in identification of promoters 1P, 3P, and 4P (referred to henceforth as 4P), respectively (Fig. 1). Transcripts from 2P or 4P were not observed in this or other experiments (Fig. 6, data not shown). Such experiments should have resolved 4P transcripts (82 or 83 nucleotides) from 4P transcripts (78 nucleotides). Radioactive products near the top of the autoradiogram (and in Fig. 6A) are presumably due to end-to-end transcription of the restriction fragment.

Run-off transcription assays were performed with the PstI-ClaI fragment to determine the influence of dnaA protein on rpoH transcription (Fig. 6, A and B). Transcription from rpoH4P was markedly inhibited with comparatively less inhibition from 3P with increasing amounts of purified dnaA protein added. At these levels of dnaA protein, specific binding of dnaA protein to the rpoH promoter region was observed (Figs. 2 and 3). Similar results of transcriptional inhibition of rpoH3P and 4P by dnaA protein were obtained with the HpaII or EcoRV DNA fragments containing the rpoH promoter region (data not shown). No inhibition by dnaA protein was observed on transcription from rpoH1P.

Run-off transcription assays were also performed with a 203-bp restriction fragment containing the lacUV5 promoter. By sequence analysis, this DNA fragment lacks sequences similar to the consensus dnaA protein recognition sequence and is poorly bound by dnaA protein (Fig. 4). The observed transcript of about 67 nucleotides is consistent with transcription from the lacUV5 promoter in the absence of cyclic AMP binding protein (Fig. 6A) (Maizels, 1973). Addition of increasing amounts of dnaA protein to these assays marginally affected the synthesis of this transcript (Fig. 6B). The product of about 240 nucleotides is presumably due to end-to-end transcription of the restriction fragment. Its slightly greater apparent size appears to be due to its anomalous electrophoretic migration in this experiment compared to other experi-

FIG. 6. dnaA protein inhibits transcription from rpoH3P and rpoH4P. A, run-off transcription assays were performed as described under “Experimental Procedures” with the 203-bp EcoRI fragment containing the lacUV5 promoter or the 1891-bp PsmI-ClaI fragment containing the rpoH promoter region. The amounts of dnaA protein added are indicated. The size standard was as in Fig. 5. B, the relative amounts of each transcript determined as described under “Experimental Procedures” are expressed as a ratio compared to the amount produced with no dnaA protein added.
ments of run-off transcription with this fragment (Wang and Kaguni, 1987; data not shown). Other high molecular weight products are unexplained but have been observed by others in transcription of this fragment (Lorimer and Revzin, 1986).

dnaA Protein Inhibits rpoH Transcription in Vivo.—The plasmid pDS596 contains the dnaA gene under inducible expression from the araB promoter (Hwang and Kaguni, 1988). In the uninduced state, transcription from the araB promoter is repressed by the araC gene product encoded by the vector pING1. Based on the above in vitro results, increased levels of dnaA protein by induced expression of the cloned dnaA gene were expected to inhibit rpoH transcription.

*E. coli* AB1157 containing either pDS596 or the vector pING1 was grown in Luria broth media at 30°C. Expression from the araB promoter was induced by addition of arabinose. Total cellular RNA was isolated from portions of each culture removed prior to and at various times after addition of arabinose. RNA was hybridized to an excess of 5’ end-labeled EcoRV restriction fragment containing the rpoH promoter region, treated with S1 nuclease, electrophoresed, and autoradiographed (Fig. 7A). The relative amounts of each transcript from the rpoH promoters were determined (Fig. 7B).

S1 mapping experiments with RNA from *E. coli* AB1157 containing the vector pING1 indicated a relatively constant level of transcript from rpoH1P prior to and at various times after addition of arabinose. Its addition appeared to result in a transient increase in 4P transcript at 10 min before returning to the original level. The reason for this transient increase is not understood. Transcripts initiating from 3P were barely detectable compared to 1P and 4P. Although transcripts from 2P were not detected, the relative levels of other rpoH transcripts prior to arabinose addition are in agreement with observations of Erickson et al. (1987) and Tobe et al. (1987). The influence of arabinose addition on 3P transcription was not determined due to its low abundance prior to arabinose addition. The S1-resistant material migrating near the bottom of the gel and near 100–130 nucleotides appeared to have arisen by hybrid formation between the radioactive probe and yeast tRNA and/or the denatured salmon sperm DNA in

![Fig. 7. Inhibition of rpoH4P transcription by overproduction of dnaA protein in vivo.](image)

A, *E. coli* AB1157 harboring either the vector pING1 or pDS596 containing the dnaA gene were grown in Luria broth media at 30°C to a turbidity at 595 nm of 0.1. Arabinose was then added to 0.75% (w/v) to induce synthesis of dnaA protein. RNA was isolated from portions of each culture removed prior to or at the indicated times after arabinose addition and used in S1 mapping experiments as described under "Experimental Procedures." The molecular weight markers were 3’ end-labeled pUC19 DNA digested with EcoRI and HpaII. B, rpoH1P and 4P transcripts were quantitated as described under "Experimental Procedures." The level of each respective transcript is expressed as a ratio compared to that contained in RNA from AB1157 harboring the vector pING1 and removed just prior to the time of arabinose addition. Open symbols (C, Δ), rpoH from AB1157 containing pING1; closed symbols (●, △), rpoH mRNA from AB1157 containing pDS596.

![Fig. 8. Inhibition of rpoH5P and rpoH4P transcription by dnaA protein after a temperature shift.](image)

A, *E. coli* AB1157 harboring either the vector pING1 or pDS596 containing the dnaA gene were grown in Luria broth media at 30°C to a turbidity at 595 nm of 0.1. Arabinose was added to 0.75% (w/v), the cultures were incubated at 30°C for 2 h and then shifted to 42°C. RNA was isolated from portions of each culture prior to or at the times indicated after the temperature shift. S1 nuclease mapping experiments were performed as described under "Experimental Procedures." The size standard was as in Fig. 7. B, rpoH1P and 4P transcripts were quantitated as described under "Experimental Procedures" and normalized to the level of each respective transcript contained in RNA from AB1157 harboring pING1 at 30°C. Open symbols (C, Δ), rpoH mRNA from AB1157 containing pING1; closed symbols (●, △), rpoH mRNA from AB1157 containing pDS596.

Inhibition of rpoH5P and rpoH4P transcription by dnaA protein after a temperature shift. A, *E. coli* AB1157 harboring either the vector pING1 or pDS596 containing the dnaA gene were grown in Luria broth media at 30°C to a turbidity at 595 nm of 0.1. Arabinose was added to 0.75% (w/v), the cultures were incubated at 30°C for 2 h and then shifted to 42°C. RNA was isolated from portions of each culture prior to or at the times indicated after the temperature shift. S1 nuclease mapping experiments were performed as described under "Experimental Procedures." The size standard was as in Fig. 7. B, rpoH1P and 4P transcripts were quantitated as described under "Experimental Procedures" and normalized to the level of each respective transcript contained in RNA from AB1157 harboring pING1 at 30°C. Open symbols (C, Δ), rpoH mRNA from AB1157 containing pING1; closed symbols (●, △), rpoH mRNA from AB1157 containing pDS596.

Figures 7 and 8. Inhibition of rpoH5P and rpoH4P transcription by dnaA protein after a temperature shift. A, *E. coli* AB1157 harboring either the vector pING1 or pDS596 containing the dnaA gene were grown in Luria broth media at 30°C to a turbidity at 595 nm of 0.1. Arabinose was added to 0.75% (w/v), the cultures were incubated at 30°C for 2 h and then shifted to 42°C. RNA was isolated from portions of each culture prior to or at the times indicated after the temperature shift. S1 nuclease mapping experiments were performed as described under "Experimental Procedures." The size standard was as in Fig. 7. B, rpoH1P and 4P transcripts were quantitated as described under "Experimental Procedures" and normalized to the level of each respective transcript contained in RNA from AB1157 harboring pING1 at 30°C. Open symbols (C, Δ), rpoH mRNA from AB1157 containing pING1; closed symbols (●, △), rpoH mRNA from AB1157 containing pDS596.

In conclusion, the relative level of 1P transcript was marginally altered by elevated levels of dnaA protein. As above, the effect of elevated levels of dnaA protein on transcription from rpoH3P was not determined due to its low abundance prior to arabinose addition.

Reports indicate that the levels of rpoH transcripts increase with an increase in temperature (Tilly et al., 1986; Erickson et al., 1987). Transcripts from 2P and 3P were observed to increase the most (Erickson et al., 1987). This increased transcription contributes, in part, to elevated expression of heat shock genes upon a temperature upshift. To determine whether elevated levels of dnaA protein would inhibit this increase in transcript levels, AB1157 containing either pDS596 or pING1 were grown at 30°C in Luria broth media, arabinose was added, and induced expression of dnaA protein proceeded for 2 h at 30°C. The culture was then shifted to 42°C. RNA was isolated from portions of each culture prior to and at various times after the temperature shift.

S1 mapping experiments with RNA from AB1157 containing the vector pING1 showed a 2- to 3-fold increase with the temperature shift in the level of rpoH1P transcripts (Fig. 8, A and B). Consistent with previous observations (Erickson et al., 1987), 3P transcripts were most dramatically elevated by the temperature shift. Due to the low levels of 3P transcript detected prior to the temperature shift, it was not possible to quantitate accurately the relative increase upon temperature increase.

2 D. S. Hwang and J. M. Kaguni, unpublished results.
upshift. In addition, an increase greater than 15-fold in the level of 4P transcript was observed 15-20 min after the temperature shift. Transcriptional start sites mapped by S1 analysis on sequencing gels for rpoH1P, 3P, and 4Pa were in agreement with Erickson et al. (1987) (data not shown). Transcripts from 4Pb were not detected. As in Fig. 7A, the S1-resistant material near the bottom of the gel and near 100-130 nucleotides were attributed to annealing between the probe and yeast tRNA and/or the denatured salmon sperm DNA included in the reactions (data not shown). Transcripts from 2P were not observed.

S1 mapping experiments with RNA isolated from AB1157 containing pDS596 indicated that rpoH1P transcripts increased to between 2- to 3-fold (Fig. 8, A and B). This result was similar to levels observed with RNA from AB1157 containing the vector. By contrast, the large increase in 3P and 4P transcripts was not observed after the temperature shift. Based on other experiments (Hwang and Kaguni, 1988), this strain presumably contains elevated levels of dnaA protein which inhibits transcription from 3P upon the temperature shift. For reasons described above, the levels of 3P transcripts were not quantitated. Although the amount of 4P transcripts increased to 3-fold with time after the temperature shift, levels of this mRNA were far less than those observed with AB1157 containing the vector pING1 at similar times. These results indicate that dnaA protein preferentially inhibits transcription from rpoH3P and 4P in vivo.

DISCUSSION

Upon a shift to high temperature, E. coli expresses a set of about 17 proteins in a phenomenon termed the heat shock response. This response appears to have been conserved in all organisms suggesting its essential role in cell physiology.

Due to the presence of two presumptive dnaA protein recognition sequences in the rpoH promoter region, we were interested in determining whether dnaA protein would specifically bind to these sites to influence rpoH expression. The results shown here indicate that dnaA protein specifically bound to the rpoH promoter region to repress transcription from promoters 3P and 4P. That transcription from 1P was not inhibited indicates that dnaA protein bound to the promoter region of 3P and 4P interferes with the binding of RNA polymerase. This differential inhibition also suggests that dnaA protein bound at a downstream site does not interfere markedly with the progress of a transcribing RNA polymerase. This result is consistent with the observation that lac repressor bound at a distal site only transiently blocks a transcriptional elongation complex (Reznikoff et al., 1989; Horowitz and Platt, 1982).

Although dnaA protein can be isolated as a monomer, its form as a transcriptional repressor remains to be determined. In studies of dnaA protein binding to the dnaA promoter region (Fuller et al., 1984; Braun et al., 1985), a complex DNase I footprint was observed which extended 45-50 bp on either side of the dnaA protein recognition sequence. This result appears to have been due to addition of excess amounts of dnaA protein. At levels of dnaA protein (2 pmol of protein with 0.025 pmol of fragment) sufficient to specifically inhibit rpoH (this study) and dnaA transcription, protection from DNase I was limited to a 30-40-bp region encompassing the dnaA protein recognition sequence. In comparison, lac repressor, a tetramer of identical subunits, protects a 25-bp region when bound to its operator (Galas and Schmitz, 1978).

The role of the heat shock response is not well understood. Proposed models relate the heat shock response to thermotolerance, protein degradation, or cell division. In E. coli, thermotolerance upon temperature upshift is dependent on a brief incubation at an elevated but nonlethal temperature (Yamamori and Yura, 1982). In this previous study, exposure of cells to this intermediate temperature was presumed necessary for expression of heat shock proteins. However, induction of the rpoH gene product under control of the inducible tac promoter at low temperature did not confer thermotolerance at high temperature (VanBogelen et al., 1987). Thermotolerance did not appear to depend on rpoH-dependent expression of heat shock proteins.

The production of abnormal proteins in E. coli by incorporation of purumycin, the arginine analog, canavanine, or by induced synthesis of a foreign protein results in elevated levels of heat shock proteins (Goff and Goldberg, 1985). Other conditions which induce the heat shock response may also generate abnormal polypeptides (Ananthan et al., 1986). Since Lon, an ATP-dependent protease (Chung and Goldberg, 1981; Charette et al., 1981), is a heat shock protein, the heat shock response may function in protein degradation. As the level of dnaA protein appears constant throughout the cell cycle (Sakikabara and Yuasa, 1982), it is unlikely that dnaA protein-dependent regulation of rpoH functions to autoregulate indirectly the level of dnaA protein by proteolysis.

Other treatments which result in expression of heat shock proteins include ethanol, UV irradiation, or bacteriophage infection. That dnaA protein negatively regulates rpoH expression suggests that these treatments may inactivate dnaA protein as a repressor or in the case of bacteriophage infection, that a site(s) on the replicating viral DNA or a viral gene product acts as a competitor for binding of dnaA protein.

The role of the heat shock response in cell division is based on studies of rpoH mutants. Whereas rpoH nonsense (and some missense) mutants are viable up to 30 °C in nonsuppressing strains due to the production of trace amounts of $\sigma^{32}$, an rpoH::kan mutant is viable only at or below 20 °C (Tobe et al., 1987). $\sigma^{32}$ appears to be essential at higher growth temperatures. When such mutants are shifted to 42 °C, cell division stops almost immediately with an increase in mass to form long filaments (Tsuchido et al., 1986).

In E. coli as in many organisms, DNA replication and cell growth are tightly coordinated events. dnaA protein functions at an early step in initiation of DNA replication from the chromosomal origin, oriC (Zyskind et al., 1977; Kung and Glaser, 1978; Bramhill and Kornberg, 1988). That expression of rpoH is influenced by this initiator protein for DNA replication and that rpoH mutants render the cell incapable of proper cell division (Tsuchido et al., 1986) suggest a regulatory mechanism whereby dnaA protein may coordinate initiation of DNA replication to the expression of genes under control of rpoH which are required for cell division.

With regard to this, an allele of dnaK, a heat shock gene, has been isolated which is conditionally defective in initiation of DNA replication (Sakikabara, 1988). We have recently determined that dnaK protein stimulates the replication activity of a mutant form of dnaA protein. dnaA protein also appears to influence the activity of wild type dnaA protein in replication. The ability of dnaA protein to regulate rpoH expression may influence the level of dnaK protein which in turn influences the activity of dnaA protein in initiation.

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