JOURNAL OF BACTERIOLOGY, July 1991, p. 4247–4253
0021-9193/91/144247-07$02.00/0

Vol. 173, No. 14

Expression of ClpB, an Analog of the ATP-Dependent Protease Regulatory Subunit in *Escherichia coli*, Is Controlled by a Heat Shock σ Factor (σ^{32})

MASANARI KITAGAWA,† CHIEKO WADA, SACHIYO YOSHIOKA, AND TAKASHI YURA*

Institute for Virus Research, Kyoto University, Kyoto 606, Japan

Received 26 February 1991/Accepted 15 April 1991

***Escherichia coli* K-12 produces at least two ATP-dependent proteases, Lon (La) and Clp (Ti), the latter consisting of a regulatory subunit (ClpA) and a proteolytic subunit (ClpP). The gene *clpB* encoding an analog of ClpA had been found at 57 min on the *E. coli* chromosome. Cloning and examination of novel heat shock promoters led us to identify a major *clpB* promoter specifically controlled by a heat shock σ factor, σ^{32} (the *rpoH* [= *htpR*] gene product). β-Galactosidase synthesis from a P*clpB-lacZ* operon fusion was transiently induced upon temperature shift from 30 to 42°C, and the induction depended on the *rpoH* function. Chromosomal *clpB* transcripts also increased upon temperature upshift and were totally absent in the *rpoH* deletion strain. In the in vitro transcription experiments, the *clpB* promoter was specifically recognized and transcribed by RNA polymerase-σ^{32}. Nucleotide sequencing and determination of mRNA start sites permitted us to identify a major heat shock promoter located upstream of the *clpB* coding sequence. The results clearly indicate that *clpB* expression is under direct control of σ^{32}. Since ClpP was recently shown to be a σ^{32}-dependent heat shock protein, the present finding suggests the possibility that a potential ATP-dependent protease, ClpB-ClpP complex, plays an important role against thermal stress in *E. coli*.**

When *Escherichia coli* cells are exposed to high temperatures, a set of well-conserved heat shock proteins is transiently induced (10, 23). Induction occurs coordinately at the level of transcription (33) and is mediated by a minor σ factor, σ^{32} (11), product of the *rpoH* (*htpR*) gene (11, 22, 34). About 20 heat shock proteins, whose expression is apparently under σ^{32} control, are induced following temperature shift from 30 to 42°C, and genes for half of them have been identified and characterized (7, 23). Among them are the GroEL and GroES proteins that play a key protective role against thermal stress (18), presumably by controlling formation and maintenance of higher-order protein structure (7, 10). DnaK, DnaJ, and GrpE proteins work synergistically in facilitating assembly and disassembly of proteins in various processes, including DNA replication of certain phages and plasmids, autoregulation of the heat shock response, and modulation of a proteolytic system (7, 10, 28). The major σ factor (σ^{70}) involved in transcription of most cellular genes is partly under σ^{32} control (29). Furthermore, ATP-dependent protease Lon (La) and a proteolytic subunit (ClpP) of another ATP-dependent protease (8) are under heat shock control mediated by σ^{32} (17, 23). Thus, many of the proteins that belong to the heat shock regulon play active roles in the synthesis of DNA, RNA, and protein and in protein degradation.

Promoters of heat shock genes recognized by RNA polymerase containing σ^{32} ($E\sigma^{32}$) exhibit characteristic sequences (at both −35 and −10 regions) that are distinct from those recognized by RNA polymerase containing σ^{70} ($E\sigma^{70}$) (5, 10). Besides the chromosomal genes related to the heat shock response, a major promoter for the replication initiator gene (*repE*) of mini-F plasmid is under the control of σ^{32} (31). The *repE* promoter has a sequence similar to that of the heat shock promoter of *rpoD* encoding σ^{70}. In addition to the σ^{32}-dependent promoters, heat-inducible promoters active at a very high temperature (50°C) have been shown to participate in transcription of *rpoH* and *htrA* (= *degP*; encoding membrane protease) (7, 10, 19). Transcription from the two promoters is specifically catalyzed by RNA polymerase containing a new σ factor, σ^{E} (6, 32), suggesting that these genes define the second heat shock regulon (7, 10).

To gain further insight into the structure and function of heat shock genes and proteins in *E. coli*, we set out to look for novel heat shock promoters using promoter-cloning vectors of low copy number. Several σ^{32}-dependent promoters isolated were analyzed in detail: they were mapped on the chromosome, nucleotide sequences were determined, and transcripts were analyzed in vivo and in vitro. One of the promoters was found to be involved in expression of *clpB*, a structural analog of *clpA* which encodes a regulatory subunit of ATP-dependent Clp protease (8). ClpA of 81 kDa has been shown to bind and activate the proteolytic subunit of 21 kDa encoded by *clpP*. The proteolytic subunit ClpP is under σ^{32} control (17), whereas the regulatory subunit (ClpA) is apparently under separate control (8). Both ClpA and ClpP are well conserved among prokaryotes and eukaryotes (9). We show in this paper that *clpB* is a novel heat shock gene whose transcription depends mostly on $E\sigma^{32}$, on the basis of the analysis of *clpB* transcription in vivo and in vitro as well as mapping and nucleotide sequence determination of the *clpB* region.

MATERIALS AND METHODS

Bacterial strains, bacteriophage, and plasmids. The bacterial strains used were derivatives of *E. coli* K-12. Strain W3110 (F^- prototroph) was used as a DNA source, and HI2017 (12) [F^- Δ(*ara-leu*)*7697* Δ(*lac-pro*) *trpA38 thy thi rpsL*] was used as a host for cloning heat shock promoters. MC4100 [F^- *araD139* Δ(*argF-lac*)*U169 rpsL150 relA1 flb-*

* Corresponding author.
† Present address: Biocenter, Takara Shuzo Co., Ltd., Otsu 520-21, Japan.

5301 deoC1 ptsF25 rbsR] (4), an isogenic temperature-sensitive *rpoH15* mutant (30), and a temperature-resistant revertant of a Δ*rpoH* mutant (18) were used for transcription studies, unless otherwise indicated. A minimum set of 476 phage λ clones covering the entire genome of *E. coli* (Kohara clones [16]) was kindly provided by K. Isono. A phage vector for cloning promoters has been described previously (12, 35). A promoter-cloning plasmid pFF6, generously supplied by M. Imai, was a derivative of mini-F carrying the *ara-trp-lac* fusion, identical to that carried by λpF13 (12).

Media and chemicals. L broth and P broth have been described previously (30). Solid media contained 1.2% agar, and ampicillin (20 to 50 μg/ml) was used for selection of drug-resistant transformants. MacConkey-lactose agar (Difco) or L agar containing X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) (60 to 80 μg/ml) was used for screening clones with heat shock promoters as well as for examining *lacZ* expression from a cloned promoter. Restriction enzymes, reverse transcriptase (Rous-associated virus type 2), and T4 polynucleotide kinase were supplied by Takara Shuzo Co., Kyoto, Japan. Nylon membranes to which a minimum set of Kohara clones (Takara Shuzo Co.) were blotted were used for mapping cloned *E. coli* DNA fragments. [α-^{32}P]dCTP (111 TBq/mmol), [γ-^{32}P]ATP (222 TBq/mmol), and [α-^{32}P]UTP (111 TBq/mmol) were obtained from Dupont, NEN Research Products (Boston, Mass.).

In vitro transcription. The reaction mixture and the conditions for RNA synthesis were essentially as described previously (31). Purified RNA polymerase holoenzymes containing σ^{32} or σ^{70} that are essentially free of each other were generous gifts of C. A. Gross (University of Wisconsin, Madison) and A. Ishihama (National Institute of Genetics, Mishima, Japan), respectively. Template DNA was prepared by amplifying the cloned promoter-containing fragment (F18; 572 bp) by polymerase chain reaction, using λpF13-(P*clpB-lacZ*) DNA and primers for *trp* and *lacZ* regions. Amplified DNAs were purified with Centricon-100 (Grace & Co., Danvers, Mass.) to remove excess nucleotides and primers. DNA fragments containing *trp* or *groE* promoter were used as control templates for transcription by Eσ^{70} or Eσ^{32}, respectively. ^{32}P-labeled RNA products were analyzed by urea-polyacrylamide gel electrophoresis as described previously (11).

Primer extension analysis of RNAs. Primer extension experiments were carried out essentially as described previously (2). Briefly, an excess (2 pmol) of synthetic primer DNA that corresponded to an N-terminal portion of *clpB* (nucleotides 119 to 144 in Fig. 4) was end labeled with [γ-^{32}P]ATP and annealed with RNAs at 65°C, and cDNA was synthesized with reverse transcriptase (Rous-associated virus type 2). The resulting cDNA was subjected to 8 M urea–6% polyacrylamide gel electrophoresis. For analysis of in vivo transcripts, cells were grown in P broth and RNAs were extracted with phenol at 65°C essentially as described previously (1); 20 μg of RNA was used for each experiment. For in vitro transcripts, product RNAs were digested with DNase I, extracted by phenol saturated with 20 mM Na-acetate (pH 5.5) at 65°C, and precipitated with ethanol at −70°C.

Other methods. Southern blot hybridization, DNA sequencing, and other recombinant DNA techniques were done by standard procedures (24). Mapping of the *E. coli* gene on the chromosome was done by DNA blot hybridization (24).

Nucleotide sequence accession number. The nucleotide sequence of the entire *Bam*HI fragment containing upstream (1,722 bp) and most (1,781 bp) of the *clpB* coding region has been deposited in the EMBL data base (accession no. X57620).

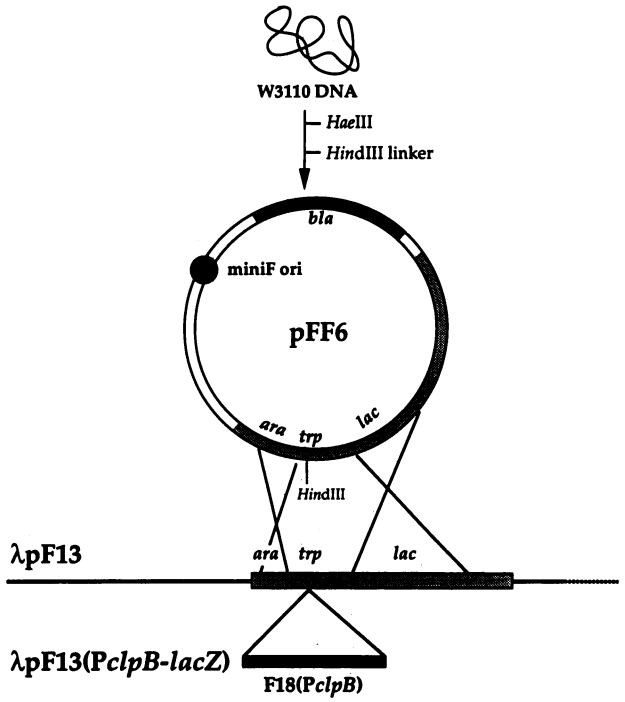

FIG. 1. Cloning of F18 fragment (*clpB* promoter) and construction of phage λpF13-(P*clpB-lacZ*). *E. coli* DNA fragments (*Hae*III digest) were first cloned into the *Hin*dIII site of plasmid pFF6 and then transferred to phage vector λpF13 by genetic recombination (12, 35). See the text for a further description of procedures.

RESULTS

Cloning of DNA fragment F18 carrying the *clpB* promoter. *E. coli* chromosomal DNA extracted from strain W3110 was partially digested with *Hae*III endonuclease and separated by agarose gel electrophoresis. DNA fragments of 400 to 600 bp were collected, ligated with *Hin*dIII linker, and inserted into the *Hin*dIII site of a promoter-cloning vector pFF6 (Fig. 1). Transformants that appeared on L agar containing ampicillin and X-Gal at 37°C were screened for blue colonies and were examined for those that exhibited enhanced *lacZ* expression upon temperature shift from 30 to 42°C. One of the clones obtained carried a DNA fragment (F18) containing a *clpB* heat shock promoter (P*clpB*), as will be shown below. The F18 fragment inserted within the *ara-trp-lac* operon fusion was then transferred to λpF13 phage carrying the same fusion by recombination in vivo, yielding λpF13-(P*clpB-lacZ*) (Fig. 1).

Dependency of *clpB* transcription on *rpoH* function. Heat inducibility of β-galactosidase synthesis was compared between the wild type (MC4100) and the *rpoH15* mutant (KY1431), each carrying λpF13-(P*clpB-lacZ*) as a prophage. In these strains, *lacZ* expression depended on a *clpB* promoter located within the cloned fragment F18. When bacteria grown at 30°C were transferred to 42°C, the rate of β-galactosidase synthesis in the wild type increased markedly and transiently (Fig. 2), with kinetics similar to those for induction of the known heat shock genes (35). The promoter

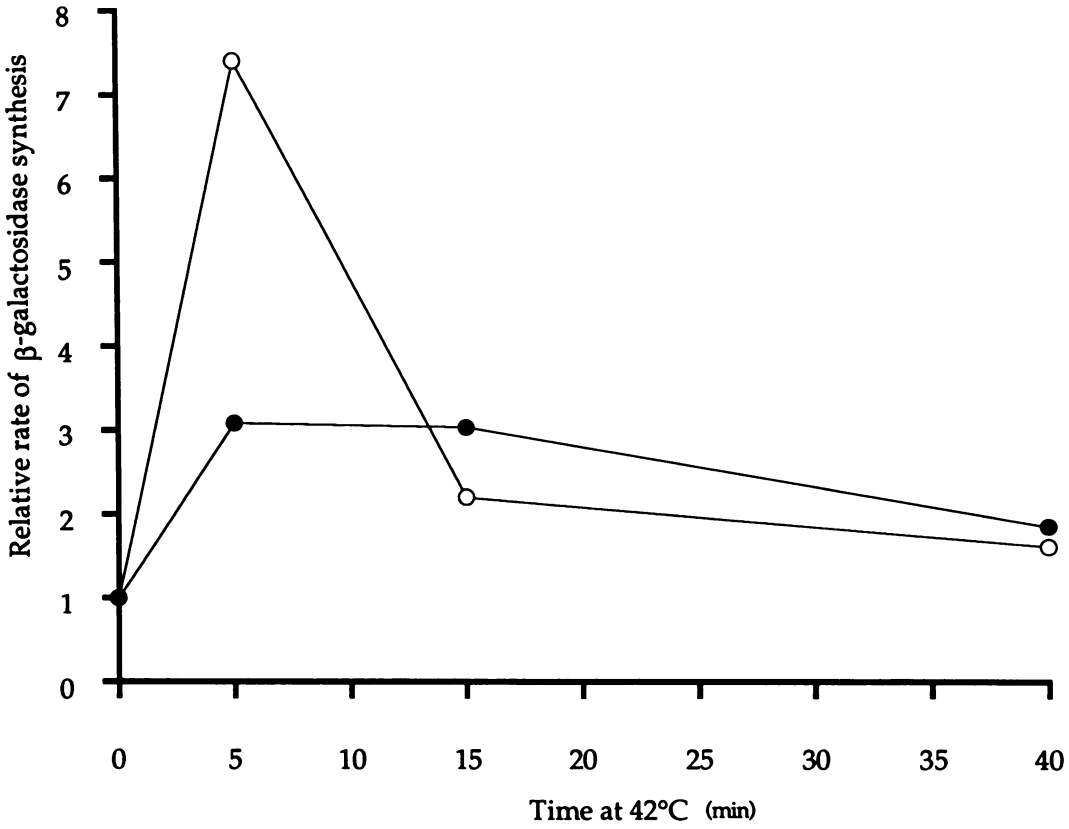

FIG. 2. Kinetics of β-galactosidase synthesis during temperature upshift. Exponential cultures of the wild type (*rpoH*$^+$) and *rpoH15* mutant lysogenic for λpF13-(P*clpB-lacZ*) were grown in P broth at 30°C and transferred to 42°C at time zero. Samples were taken at 0, 10, 20, and 60 min and assayed for β-galactosidase activity as described previously (35). The increase in enzyme activity for each time interval was divided by the corresponding increase in optical density and was normalized to the zero time value for each strain. The result of a typical experiment was plotted at the midpoint of each interval. The rate of enzyme synthesis at 30°C was ca. 100 and 28 U (21) for the wild type and the mutant, respectively. The value for the phage vector (λpF13) without the promoter inserted was 0.2 U. ○, MC4100 (*rpoH*$^+$); ●, KY1431 (*rpoH15*).

activity as judged by β-galactosidase activity was comparable to that for the *groE* heat shock promoter. In contrast, the enzyme level in the *rpoH15* mutant was lower (three- to fourfold) than in the wild type at 30°C, and relatively little induction occurred upon shift to 42°C. The results indicate that transcription from a putative *clpB* promoter (or promoters) depends on *rpoH* function directly or indirectly. In agreement with these results, primer extension analysis of transcripts of *clpB-lacZ* operon fusion by using an appropriate probe DNA revealed a few abundant heat-inducible transcripts initiated from within the F18 fragment. These transcripts could not be detected in RNAs from the Δ*rpoH* strain lacking σ^{32} (see below; Fig. 6).

Mapping of the *clpB* promoter(s) on the chromosome. To map the *clpB* promoter(s) on the *E. coli* chromosome, the F18 DNA was labeled with [γ-^{32}P]ATP and hybridized with a set of *E. coli* ordered clones (16) that had been blotted onto nylon membranes. Clone 21D7 specifically hybridized with the probe DNA, indicating that the *clpB* promoter originated from the 57-min region of the chromosome. The chromosomal DNA segment of clone 21D7 was isolated and digested with each of the eight restriction enzymes used originally in construction of the clones. The DNA digests were separated by agarose gel electrophoresis, transferred to a nylon membrane, and hybridized with ^{32}P-labeled F18 DNA. This permitted us to localize the F18 fragment on the restriction map (16) shown in Fig. 3, in which one of the *Eco*RI sites reported previously was not found in the present study.

Nucleotide sequence of the *clpB* promoter region. The nucleotide sequence of F18 DNA was initially determined, and a putative promoter was localized within the fragment. The entire sequence of a *Bam*HI fragment (3.5 kb; Fig. 3) derived from clone 21D7 was then determined by using a series of deletions (in both directions) derived from pUC118 carrying the fragment. We thus identified a putative heat shock promoter with a sequence similar to the σ^{32} consensus (5, 10) for both the −35 and −10 regions (Fig. 4), followed by a ribosome-binding (Shine-Dalgarno) sequence and an open reading frame. In the search for the sequence homology by using the GenBank data base, the putative open reading frame was found to be identical (except for a few nucleotides) with part of the *clpB* gene encoding an analog of the ATP-dependent protease regulatory subunit (96 kDa) (26).

Transcription of *clpB* in vitro. To directly analyze transcripts initiated from the *clpB* promoter(s), we carried out in vitro transcription experiments using F18 (*clpB*) DNA as a template and purified RNA polymerase containing either σ^{32} ($E\sigma^{32}$) or σ^{70} ($E\sigma^{70}$). A major RNA transcript with an expected size (ca. 230 nucleotides) was produced when $E\sigma^{32}$ was used under standard conditions (Fig. 5). In contrast, no

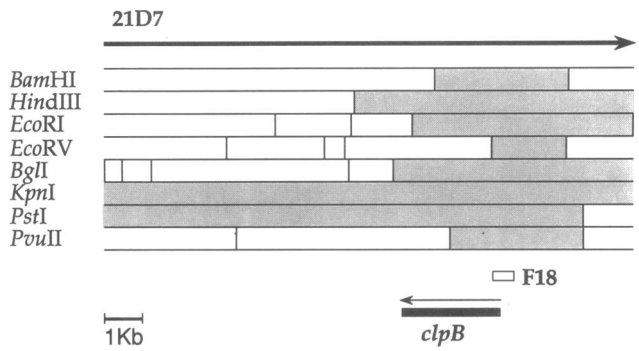

FIG. 3. Localization of the F18 (*clpB*) fragment on the physical map of Kohara clone 21D7. The map was redrawn on the basis of the published restriction map (16). Vertical bars indicate sites for each of the eight restriction enzymes as shown. The shaded restriction fragments are those which showed positive hybridization with the F18 fragment; the location of F18 DNA deduced from this and subsequent sequencing data is indicated by an open bar. The solid bar shows the *clpB* coding region, and the arrow indicates the direction of transcription.

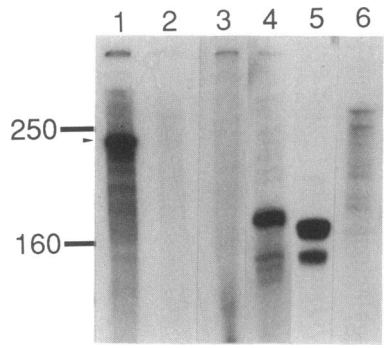

FIG. 5. Transcription of the *clpB* promoter region in vitro. RNA was synthesized with highly purified $E\sigma^{32}$ or $E\sigma^{70}$, by using F18 (*clpB*) or *trp* DNA as a template under the standard conditions, as described in Materials and Methods. ^{32}P-UTP was used as the labeled substrate. RNA was precipitated with ethanol and analyzed by urea-polyacrylamide gel electrophoresis. Arrowheads indicate major transcripts, and numbers to the left indicate the positions of size markers (in nucleotides). Lanes: 1, *clpB* DNA, $E\sigma^{32}$; 2, *clpB* DNA, $E\sigma^{70}$; 3, *trp* DNA, $E\sigma^{32}$; 4, *trp* DNA, $E\sigma^{70}$; 5, *groE* DNA, $E\sigma^{32}$; 6, *groE* DNA, $E\sigma^{70}$.

appreciable amounts of transcripts could be detected with $E\sigma^{70}$ under the conditions used. In control experiments, *trp* and *groE* transcripts were produced specifically with $E\sigma^{70}$ or $E\sigma^{32}$, respectively. These results indicate that $E\sigma^{32}$, and not $E\sigma^{70}$, can transcribe the *clpB* promoter; no accessory factors are required for transcription of this promoter by $E\sigma^{32}$ under the conditions used.

Determination of 5′ ends of *clpB* transcripts. Transcription start sites for *clpB* mRNA(s) were determined by the procedure of primer extension. RNA transcribed from F18 DNA in vitro by $E\sigma^{32}$ was first shown to initiate from a unique nucleotide A (nucleotide 1) (Fig. 6), in good agreement with the location of a heat shock promoter expected from the nucleotide sequence (Fig. 4). Cellular *clpB* mRNA was then analyzed with RNAs from wild-type and mutant cells lacking σ^{32}, grown at 30°C or for 10 min after the shift to 42°C. Major transcripts from the wild type (initiated at A or G [nucleotides 1 or 2]) coincided well with those synthesized in vitro, indicating that this represents the major *clpB* transcript(s) initiated from the heat shock promoter.

A few minor bands were found for the wild type specifically after temperature upshift, above and below the major band(s); they probably reflect transcripts from a minor promoter(s) or products of RNA processing or degradation. The nature of these bands has not been examined further. In addition, a very weak *rpoH*-independent and temperature-independent transcript initiating from nucleotide −90 was constantly detected (Fig. 4). Similar patterns of transcripts were obtained with strain W3110 or with MC4100 lysogenic

```
-367  CCGGCAATTGGTCCACGCGCGTTCGAAGTGGGGGGGGAGGTTCGCGAGGCGTTTATGGCAGTAG  -304

-303  ACGCTAAAGCAAGTGCAGCTTTCATTCAGCATGGTGATAAGTATCTGGCGGATATTTATCAGCT  -240

-239  TGCCCGGCAGCGTCTGGCGAACGTGGGTGTTGAGCAAATTTTCGGCGGCGACCGTTGTACATAT  -176

-175  ACGGAAAATGAGACTTTCTTCTCTTATCGTCGCGACAAGACCACCGGTCGTATGGCAAGTTTCA  -112

-111  TTTGGCTGATATAACCTAAAGAATCAAGACGATCCGGTACGCGTGATTTTCTTTTCACATTAAT   -48

                 -35                    -10        +1
 -47  CTGGTCAATAACCTTGAATAATTGAGGGATGACCTCATTTAATCTCCAGTAGCAACTTTGATCC    17
        TCTC-CCCTTGAA                  CCCCAT-TA        ↳mRNA

  18  GTTATGGGAGGAGTTATGCGTCTGGATCGTCTTACTAATAAATTCCAGCTTGCTCTTGCCGATG    81
                    M  R  L  D  R  L  T  N  K  F  Q  L  A  L  A  D

  82  CCCAATCACTTGCACTCGGGCACGACAACCAATTTATCGAACCACTTCATTTAATGAGCGCCCT   145
      A  Q  S  L  A  L  G  H  D  N  Q  F  I  E  P  L  H  L  M  S  A  L

 146  GCTGAATCAGGAAGGGGGTTCGGTTAGTCCTTTATTAACATCCGCTGGCATAAATGCTGG       205
        L  N  Q  E  G  G  S  V  S  P  L  L  T  S  A  G  I  N  A
```

FIG. 4. Nucleotide sequence of the *clpB* promoter region. The sequence of F18 DNA containing the *clpB* promoter and an N-terminal portion of the *clpB* coding region is shown. Nucleotide +1 indicates the start site of the major transcript found in both in vivo and in vitro experiments (see text). The −35 and −10 regions are compared with the σ^{32} consensus sequence shown below (matched nucleotides are shown by boldface type) (10). A sequence of rotational symmetry and a putative ribosome binding site are overlined. The amino acid sequence predicted from the nucleotide sequence is shown below the nucleotide sequence. A sequence complementary to the primer DNA used for primer extension analysis of RNAs (see Fig. 6) is underlined.

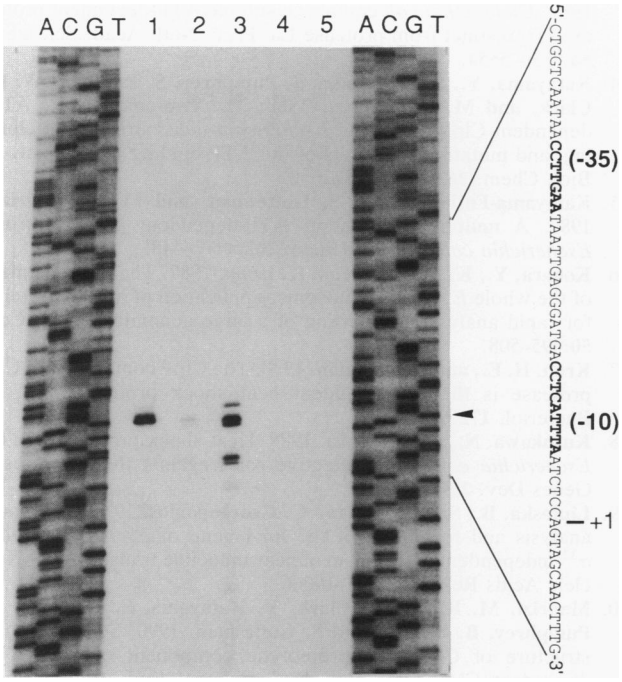

FIG. 6. Primer extention analysis of *clpB* transcripts. Transcription start sites for RNAs synthesized both in vitro and in vivo were determined by the primer extension procedure, by using an oligonucleotide primer (within the *clpB* coding region, shown in Fig. 4) as described in Materials and Methods. DNA sequence ladders were generated by the dideoxy-chain termination method, using F18 DNA as a template and the same primer used for the primer extention reaction. In vitro RNA was synthesized by $E\sigma^{32}$ as in Fig. 5, except that no labeled substrate was used. Cellular RNAs were extracted from cultures grown at 30°C or for 10 min after the shift to 42°C. Lane 1, RNA synthesized in vitro. Lanes 2 to 5, RNAs extracted from cells under following conditions: 2, MC4100 (*rpoH*$^+$), 30°C; 3, MC4100 (*rpoH*$^+$), 42°C; 4, KY1603 (Δ*rpoH*), 30°C; 5, KY1603 (Δ*rpoH*), 42°C. The nucleotide sequence of the *clpB* promoter region is indicated to the right; an arrowhead indicates the start site of major RNA produced both in vivo and in vitro.

for λpF13-(P*clpB-lacZ*) (data not shown). No appreciable amounts of read-through transcripts could be detected under the conditions of the present experiments. All results taken together lead us to conclude that *clpB* transcription is mainly initiated from a heat shock promoter by RNA polymerase containing σ^{32} both in vivo and in vitro.

DISCUSSION

Examination of heat-inducible promoters cloned from random *E. coli* DNA fragments led us to find that *clpB* transcription primarily depends on the heat shock promoter recognized by $E\sigma^{32}$. First, expression of the P*clpB-lacZ* operon fusion depended on the *rpoH* function and was markedly enhanced upon temperature shift from 30 to 42°C (Fig. 2). Second, the *clpB* mRNA level in wild-type cells increased after temperature upshift, whereas little or no mRNA was detected in the Δ*rpoH* mutant at either temperature (Fig. 6). Third, the *clpB* promoter contained a sequence highly homologous to that of other σ^{32}-specific promoters (10) (Fig. 4). Finally, the *clpB* promoter was specifically recognized and transcribed by $E\sigma^{32}$ in vitro (Fig. 5). The novel *clpB* promoter defined by the present study is quite active at high temperatures and is comparable to the *groE* promoter, on the basis of β-galactosidase activity (Fig. 2) and the amount of *clpB* mRNA detected by the primer extention experiments (data not shown).

Some minor *clpB* transcripts were found with mRNAs prepared from heat-shocked wild-type bacteria. One of the bands, seen 3 nucleotides above the major band in Fig. 6, may reflect a transcript driven by the same (major) promoter, considering the proximity and sequence of the promoter-start site region. On the other hand, twin bands detected 8 to 9 nucleotides below the major band may reflect products of processing or degradation of major *clpB* mRNA, although possible involvement of a separate nearby heat shock promoter is not excluded. In any event, transcription of *clpB* is primarily mediated by $E\sigma^{32}$ and can be regulated by temperature and cellular concentration of σ^{32} like that of the other heat shock genes. This, however, does not exclude the possibility that *clpB* transcription is also controlled by other environmental factors. In this connection, it is interesting to find a sequence (29 bp) of rotational symmetry covering both −35 and −10 regions of the *clpB* major promoter (Fig. 4); this might play an important role by interacting with some unidentified regulatory protein. Immediately following the *clpB* coding region is a rho-independent transcriptional terminator and an rRNA operon (*rrnG*) (25). It is unlikely, though not excluded, that the heat shock control of *clpB* transcription extends to the downstream *rrnG*.

ATP-dependent protease Lon has been known to be involved in degradation of a variety of abnormal proteins. Synthesis of Lon protease is under the control of σ^{32} and is markedly enhanced when exposed to higher temperatures. This suggests that Lon plays a protective role against thermal stress by degrading excessive abnormal proteins. Since *rpoH* mutants are defective in the breakdown of abnormal proteins to a greater extent than are the *lon* mutants (3), at least one proteolytic system other than Lon is missing in *rpoH* mutants (8). Also, *lon* deletion mutants still exhibit energy-dependent degradation of unstable or abnormal proteins. In this connection, mutations affecting DnaK, DnaJ, and GrpE heat shock proteins bring about deficiencies in protein degradation, suggesting that these proteins are involved in modulating proteolysis directly or indirectly (27).

A two-component ATP-dependent Clp (Ti) protease (13, 15) found in *E. coli* might play a protective role against temperature stress, because its catalytic subunit ClpP was recently shown to be a σ^{32}-dependent heat shock protein (17). Heat-inducible *clpP* transcription is thought to be mediated by $E\sigma^{32}$, though this has not been demonstrated directly. The published nucleotide sequence suggests the possible involvement of a heat shock promoter upstream of *clpP* (TGTTATGCTTGAA-15 bp-ACCCATAAC, starting at nucleotide 264 of Fig. 6 of reference 20). Contrary to the putative role of Clp protease against thermal stress, however, synthesis of the regulatory subunit ClpA that binds stoichiometrically to ClpP is not induced by heat shock (14) but is increased under anaerobic conditions (cited in reference 8). The present finding that ClpB, unlike ClpA, is under σ^{32} control raises the interesting possibility that a ClpB-ClpP complex rather than the ClpA-ClpP protease plays a major role under heat shock conditions. The high homology found between ClpA and ClpB (9) is consistent with the notion that both can serve as regulatory subunits of Clp proteases, although to our knowledge, properties of ClpB protein have not been reported. The hypothetical ClpB-ClpP protease, if confirmed by direct experiments, would be an alternative

form of Clp proteases with different properties such as substrate specificity. It is tempting to speculate that the differential regulation of ClpA and ClpB reflects differential roles of alternative forms of energy-dependent Clp proteases whose syntheses are enhanced under respective physiological or environmental conditions.

ACKNOWLEDGMENTS

We are grateful to C. Gross, M. Imai, A. Ishihama, and K. Isono for kind gifts of RNA polymerase, plasmids, and phages used in this study. We also thank H. Mori for valuable advice in computer analysis and M. Mihara and T. Tanaka for technical assistance.

This work was supported in part by grants from the Ministry of Education, Science and Culture, Japan.

ADDENDUM

After this paper was submitted, we learned that C. L. Squires and collaborators have obtained results that are in agreement with ours. These results are presented in the accompanying paper (26a).

REFERENCES

1. **Aiba, H., S. Adhya, and B. de Crombrugghe.** 1981. Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. J. Biol. Chem. **256**:11905–11910.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1987. Primer extension, p. 4.8.1.–4.8.3. *In* Current protocols in molecular biology. Wiley Interscience, New York.
3. **Baker, T. A., A. D. Grossman, and C. A. Gross.** 1984. A gene regulating the heat shock response in *Escherichia coli* also affects proteolysis. Proc. Natl. Acad. Sci. USA **81**:6779–6783.
4. **Casadaban, M. J.** 1976. Transposition and fusion of the *lac* genes to selected promoters in *Escherichia coli* using bacteriophage lambda and Mu. J. Mol. Biol. **104**:541–556.
5. **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.
6. **Erickson, J. W., and C. A. Gross.** 1989. Identification of the σ^E subunit of *Escherichia coli* RNA polymerase: a second alternate σ factor involved in high temperature gene expression. Genes Dev. **3**:1462–1471.
7. **Georgopoulos, C., D. Ang, K. Liberek, and M. Zylicz.** 1990. Properties of the *Escherichia coli* heat shock proteins and their role in bacteriophage λ growth, p. 191–221. *In* R. I. Morimoto, A. Tissieres, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Gottesman, S.** 1989. Genetics of proteolysis in *Escherichia coli*. Annu. Rev. Genet. **23**:163–198.
9. **Gottesman, S., C. Squires, E. Pichersky, M. Carrington, M. Hobbs, J. S. Mattick, B. Dalrymple, H. Kuramitsu, T. Shiroza, T. Foster, W. P. Clark, B. Ross, C. L. Squires, and M. R. Maurizi.** 1990. Conservation of the regulatory subunit for the Clp ATP-dependent protease in prokaryotes and eukaryotes. Proc. Natl. Acad. Sci. USA **87**:3513–3517.
10. **Gross, C. A., D. B. Straus, J. W. Erickson, and T. Yura.** 1990. The function and regulation of heat shock proteins in *Escherichia coli*, p. 167–189. *In* R. I. Morimoto, A. Tissieres, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
11. **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.
12. **Hirano, M., K. Shigesada, and M. Imai.** 1987. Construction and characterization of plasmid and lambda phage vector systems for study of transcriptional control of *Escherichia coli*. Gene **57**:89–99.
13. **Hwang, B. J., W. J. Park, C. H. Chung, and A. L. Goldberg.** 1987. *Escherichia coli* contains a soluble ATP-dependent protease (Ti) distinct from protease La. Proc. Natl. Acad. Sci. USA **84**:5550–5554.
14. **Katayama, Y., S. Gottesman, J. Pumphrey, S. Rudikoff, W. P. Clark, and M. R. Maurizi.** 1988. The two-component, ATP dependent Clp protease of *Escherichia coli*: purification, cloning, and mutational analysis of the ATP-binding component. J. Biol. Chem. **263**:15226–15236.
15. **Katayama-Fujimura, Y., S. Gottesman, and M. R. Maurizi.** 1987. A multiple-component, ATP-dependent protease from *Escherichia coli*. J. Biol. Chem. **262**:4477–4485.
16. **Kohara, Y., K. Akiyama, and K. Isono.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50**:495–508.
17. **Kroh, H. E., and L. D. Simon.** 1990. The ClpP component of Clp protease is the σ^{32}-dependent heat shock protein F21.5. J. Bacteriol. **172**:6026–6034.
18. **Kusukawa, N., and T. Yura.** 1988. Heat shock protein GroE of *Escherichia coli*: key protective roles against thermal stress. Genes Dev. **2**:874–882.
19. **Lipinska, B., S. Sharma, and C. Georgopoulos.** 1988. Sequence analysis and regulation of the *htrA* gene of *Escherichia coli*: σ^{32}-independent mechanism of heat-inducible transcription. Nucleic Acids Res. **16**:10053–10066.
20. **Maurizi, M. R., W. P. Clark, Y. Katayama, S. Rudikoff, J. Pumphrey, B. Bowers, and S. Gottesman.** 1990. Sequence and structure of ClpP, the proteolytic component of the ATP-dependent Clp protease of *Escherichia coli*. J. Biol. Chem. **265**:12536–12545.
21. **Miller, J. H.** 1972. Experiments in molecular genetics, p. 352–355. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
22. **Neidhardt, F. C., and R. A. VanBogelen.** 1981. Positive regulatory gene for temperature-controlled proteins in *Escherichia coli*. Biochem. Biophys. Res. Commun. **100**:894–900.
23. **Neidhardt, F. C., and R. A. VanBogelen.** 1987. Heat shock response, 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, vol. 2. American Society for Microbiology, Washington, D.C.
24. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Shen, W.-F., C. Squires, and C. L. Squires.** 1982. Nucleotide sequence of the *rrnG* ribosomal RNA promoter region of *Escherichia coli*. Nucleic Acids Res. **10**:3303–3313.
26. **Squires, C., B. M. Ross, and C. L. Squires.** 1990. GenBank access no. M29364.

26a. **Squires, C. L., S. Pedersen, B. M. Ross, and C. Squires.** 1991. ClpB is the *Escherichia coli* heat shock protein F84.1. J. Bacteriol. **173**:4254–4262.

27. **Straus, D., W. Walter, and C. A. Gross.** 1990. DnaK, DnaJ, and GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of σ^{32}. Genes Dev. **4**:2202–2209.
28. **Straus, D. B., W. A. Walter, and C. A. Gross.** 1988. *Escherichia coli* heat shock gene mutants are defective in proteolysis. Genes Dev. **2**:1851–1858.
29. **Taylor, W. E., D. B. Straus, A. D. Grossman, Z. F. Burton, C. A. Gross, and R. R. Burgess.** 1984. Transcription from a heat-inducible promoter causes heat shock regulation of the sigma subunit of *E. coli* RNA polymerase. Cell **38**:371–381.
30. **Tobe, T., K. Ito, and T. Yura.** 1984. Isolation and physical mapping of temperature-sensitive mutants defective in heat-shock induction of proteins in *Escherichia coli*. Mol. Gen. Genet. **195**:10–16.
31. **Wada, C., M. Imai, and T. Yura.** 1987. Host control of plasmid replication: requirement for the σ factor σ^{32} in transcription of mini-F replication initiator gene. Proc. Natl. Acad. Sci. USA **84**:8849–8853.
32. **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.
33. **Yamamori, T., and T. Yura.** 1980. Temperature-induced synthesis of specific proteins in *Escherichia coli*: evidence for transcriptional control. J. Bacteriol. **142**:843–851.
34. **Yamamori, T., and T. Yura.** 1982. Genetic control of heat-shock protein synthesis and its bearing on growth and thermal resistance in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **79**:860–864.
35. **Yano, R., M. Imai, and T. Yura.** 1987. The use of operon fusions in studies of the heat-shock promoter function in *Escherichia coli*. Mol. Gen. Genet. **207**:24–28.