The *Escherichia coli* *htrP* Gene Product Is Essential for Bacterial Growth at High Temperatures: Mapping, Cloning, Sequencing, and Transcriptional Regulation of *htrP*

SATISH RAINA,* LEANNE MABEY, AND COSTA GEORGOPOULOS

Department of Cellular, Viral and Molecular Biology, University of Utah
School of Medicine, Salt Lake City, Utah 84132

Received 26 April 1991/Accepted 24 July 1991

We identified and characterized a new *Escherichia coli* gene, *htrP*. The *htrP* gene was identified because its insertional inactivation by the Tn10 transposon results in the inability of *E. coli* to form colonies at temperatures above 37°C and a slow growth phenotype at 30°C. The *htrP* gene was cloned and mapped to 66.3 min on the *E. coli* genetic map, 4 kbp clockwise from the *tolC* gene. The *htrP* gene was sequenced and shown to code for an acidic, 27,471-Da polypeptide and to be transcribed counterclockwise with respect to the genetic map. The predicted HtrP protein has two potential transmembrane segments and shares an identity of 64.4% over a length of 210 amino acids with the LuxH protein. Despite the fact that the *htrP* gene is essential for *E. coli* growth exclusively at high temperatures, the levels of *htrP*-specific transcripts decrease with increasing temperature.

Escherichia coli can survive and grow within a wide range of temperatures (10). To adapt to such a diverse range of growth temperature conditions, a variety of cellular processes are involved that allow *E. coli* to efficiently perform metabolic functions without losing viability (10). A sudden shift-up in temperature is known to induce the accelerated synthesis of a set of proteins, collectively known as heat shock proteins. The transient elevated expression of these proteins is highly conserved and is referred to as the heat shock response (22). It has been suggested that the heat shock response is part of the adaptive tolerance mechanism required for survival at high temperatures (37). Besides the induction of heat shock proteins, certain other functions are also affected. These include changes in membrane protein composition and lipid composition, transient inhibition of cell division, as well as altered turnover of many other proteins (10, 20). To gain an understanding of genes that may be uniquely required by *E. coli* to survive at high temperatures, we screened libraries of various transposon insertion mutations. Those insertion mutations that enable *E. coli* to grow well at low temperatures but result in inability to form colonies at high temperature should potentially define functions that are uniquely involved in bacterial survival at high temperature. Using this approach, we have defined thus far four such high temperature requirements (*htr*) genes. Of these, *htrA* and *htrC* define two new heat shock genes. The *htrA* gene encodes a periplasmic endopeptidase (17, 18) and is transcribed by the $E\sigma^{24}$ RNA polymerase (6). The *htrC* gene is under the transcriptional regulation of the $E\sigma^{32}$ RNA polymerase, similar to other classical heat shock genes (27). The *htrB* gene is transcribed by the $E\sigma^{70}$ RNA polymerase, which transcribes housekeeping genes (10). The *htrM* gene has at least two promoters, one transcribed by $E\sigma^{70}$ and another transcribed by $E\sigma^{32}$ (28). Here, we present the identification, mapping, and sequence analysis of a fifth *htr* gene, designated *htrP*.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are described in Table 1. The inability to form colonies at high temperatures is referred to as the temperature-sensitive (Ts^-) phenotype.

Bacteriophages. Bacteriophage λ 1105 mini-Kan::Tn10 (35) was used to insert ionally inactivate the *htrP* gene, as previously described (17, 27). Phage P1L4, used in transductions, was from our collection. The *E. coli* genomic library, prepared in the λ 2001/EMBL4 vector, was obtained from Y. Kohara (14).

Media. The LB rich and M9 minimal media were prepared as described by Maniatis et al. (19). LA is LB medium containing 1% agar. M9 medium was supplemented with glucose (0.2%), thiamine (2 μ g/ml), $MgSO_4$ (1 mM), $MgCl_2$ (3 mM), $CaCl_2$ (0.1 mM), and $FeCl_3$ (0.3 μ M). For [35 S]methionine labeling experiments, the M9 high-sulfur medium, supplemented with a mixture of defined amino acids, was used as previously described (34). When necessary, the media were supplemented with ampicillin (100 μ g/ml), tetracycline (15 μ g/ml), spectinomycin (50 μ g/ml), or kanamycin (50 μ g/ml).

Enzymes. Restriction enzymes, T4 DNA ligase, and exonuclease III were purchased from Bethesda Research Laboratories. Sequenase enzyme was purchased from United States Biochemical Corporation.

Cloning of *htrP* gene. A library of wild-type *E. coli* chromosomal DNA, digested with *Pst*I and ligated in the corresponding site of the low-copy, spectinomycin-resistant (Spc^r) vector pGB2 (4), was used to isolate wild-type *htrP* clones. The previously described *E. coli* chromosomal DNA library, prepared in cosmid vector pREG153 (23), was also used to isolate cosmids carrying the *htrP*⁺ gene. The abilities to complement the Ts^- phenotype and recombine *htrP* mutations onto the plasmid were taken as preliminary evidence that the clone carried the bona fide *htrP* gene. The gene was further subcloned by standard DNA manipulation techniques (19). All initial subcloning was done with pGB2 as the vector.

* Corresponding author.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Reference or source
<i>E. coli</i>		
B178	W3110 <i>galE</i>	8
MC4100	$\Delta(\arg F-lac)U169$ <i>araD139</i> <i>rpsL150 deoC1 relA1</i> <i>ptsF25 ffbB5501 rbsR</i>	3
JC8679	<i>thr-1 leu-6 thi-1 lacY1 galK2</i> <i>ara-14 xyl-5 proA2 his-4</i> <i>argE3 rpsL31 tsx-33 mtl-1</i> <i>recB21 recC22 sbcA23 λ^-</i> <i>F⁻ supE</i>	A. J. Clark
DH5 α	F ⁻ ϕ 80 $\Delta lacZM15$ <i>recA1</i>	Bethesda Research Laboratories
SR589	B178 <i>htrP698::Tn10</i>	This work
SR603	B178 <i>htrP510::ΩKan</i>	This work
SR407	KY1621 <i>zhf::Tn10 rpoH⁺</i>	27
SR940	MC4100(pSR723)	This work
KY1621	<i>rpoH30^A::Kan^r</i>	38
CAG12184	MG1655 <i>tolC210::Tn10</i>	31
Plasmids		
pGB2	4-kb <i>Spc^r</i> low-copy-number vector	4
pREG	Cosmid cloning vector	15, 23
pRS528	Operon fusion vector	30
pSR589	pGB2 with 6.5-kb <i>Pst</i> I fragment carrying <i>htrP⁺</i>	This work
pSR590	pGB2 with 3.5-kb <i>Pst</i> I- <i>EcoRV</i> fragment carrying <i>htrP⁺</i>	This work
pSR591	pGB2 with 1.5-kb <i>Cla</i> I- <i>Nde</i> I fragment carrying <i>htrP⁺</i>	This work
pSR592	pGB2 with 2-kb <i>Asu</i> II- <i>Ava</i> I fragment carrying truncated <i>htrP</i> gene	This work
pSR603	pSR590 (<i>htrP510::ΩKan</i>)	This work
pSR604	pSR590 (<i>htrP698::Tn10 Kan</i>)	This work
pSR723	pRS528 (<i>htrP-lacZ</i>) carrying 536-bp <i>Aat</i> II- <i>Asu</i> II fragment containing <i>htrP</i> promoter sequences	This work

Mapping of *htrP* gene. Plasmid pSR589 DNA (*htrP⁺*) or its various subclones carrying the *htrP* gene were nick translated with [α -³²P]dCTP (3,000 Ci/mmol; Dupont, NEN Research Products) and used to probe the λ *E. coli* genomic library as previously described (17).

Mapping of *Kan::Tn10* insertion in *htrP* gene. The exact nucleotide position of the *Tn10* insertion in *htrP698::Tn10* was determined by first isolating clones carrying the *htrP* mutant allele. To do this, chromosomal DNA from *htrP* mutant bacteria was digested to completion with *Pst*I. The digested DNA was ligated to pGB2 and pSK⁺ (Bluescript; Stratagene) plasmids, similarly digested with *Pst*I, and used to transform *E. coli* DH5 α to kanamycin resistance (Kan^r). The plasmid DNA from such Kan^r clones was compared by restriction enzyme digestion with pSR589 (*htrP⁺*), placing the *Tn10* insertion between the unique *Bgl*II and *Nde*I sites. To identify the junction between the chromosomal DNA and the *Kan::Tn10* insertion, we used plasmid pSR604, carrying the region from the *Hind*III site of *Kan::Tn10* (35) to the *Bgl*II site of the *htrP* gene, to sequence the junction by using both the universal reverse primer from the *Bgl*II site and a 24-nucleotide primer which reads out from the ends of the *Tn10* insertion element. The sequence of this oligonucleotide was 5'-ATTGATCATATGACAAGATGTGT-3' (36).

Construction of *htrP:: Ω Kan^r* insertion mutation. An additional *htrP* null allele was constructed by in vitro insertion of the kanamycin resistance cassette, the Ω Kan^r element (7, 26). To achieve this, we excised the 2.1-kb kanamycin resistance element of plasmid pHP45 Ω Kan^r by digestion with *Bam*HI and inserted it into the unique *Bgl*II site of pSR589 within the *htrP* coding region (Fig. 1). The resulting plasmid, pSR603, was used to transfer the mutant *htrP* allele to the chromosome by linear transformation of the *recB recC sbcA* strain JC8679 (27). Ampicillin-sensitive kanamycin-resistant transformants were retained and transferred by P1 transduction back to a wild-type *E. coli* background. The closely linked Tet^r marker from CAG12184 (*tolC210::Tn10*) was used to further verify that the new *htrP* allele was located at its expected map location.

RNA isolation and Northern (RNA) blots. RNA was isolated by the hot-phenol extraction procedure (19). RNA was

FIG. 1. Restriction map of the *htrP* gene and construction of *htrP* insertion derivatives. Restriction maps of (a) pSR589, (b) pSR590, and (c) pSR591 carrying the intact *htrP* gene; (d) map of pSR592, carrying the 2-kbp *Asu*II-*Ava*I fragment lacking the *htrP* promoter and 38 bp upstream of the AUG codon; (e) in vitro insertion of the Ω -Kan^r cassette at the *Bgl*II site of the *htrP* gene; and (f) the location and orientation of the *Tn10* insertion in the *htrP* gene.

isolated from cells grown at 30°C and from cells which were shifted to 42 or 50°C for the desired time. Northern blots (5) of total RNA (5 to 10 µg per lane) were probed with 200 ng of the radiolabeled 1.6-kbp *ClaI-NdeI* DNA fragment, carrying the entire *htrP* coding sequence, isolated from plasmid pSR591. Labeling was done by nick translation, using [α -³²P]dCTP (3,000 Ci/mmol; NEN) (19).

Construction of *htrP-lacZ* fusions. To clone the promoter region of the *htrP* gene, we cloned the 830-bp *ClaI-AsuII* fragment from pSR590 into the SK⁺ vector (Bluescript) at the *ClaI* site, resulting in plasmid pSR712. The direction of the cloned fragment in pSR712 was verified by sequencing, and then the insert was transferred to the *lacZ* operon fusion vector pRS528 (30). This was done by using the *HincII* and *EcoRI* flanking restriction sites from the vector to reisolate the *ClaI-AsuII* promoter-containing fragment and clone it into the *SmaI* and *EcoRI* sites of pRS528. This orientation of cloning resulted in an in-frame *lacZ* operon fusion. To reduce the amount of upstream sequences, we constructed a smaller clone containing only 536 bp upstream of the *AsuII* site (Fig. 1). The plasmid pSR712 was digested with *AatII* and *ClaI* and then treated with T4 DNA polymerase to make the ends blunt. The reaction mixture was then treated with T4 DNA ligase and used to transform DH5 α . The new construct, pSR723, was used to reisolate the *htrP* promoter-containing *AatII-AsuII* fragment by digesting with *HincII-EcoRI* and cloning into the pRS528 vector. The Lac⁺ constructs pRS528(*htrP-lacZ*)830 and pRS528(*htrP-lacZ*)536 were first verified in strain DH5 α and then subsequently used to transform MC4100 to the Lac⁺ phenotype. To make single-copy *htrP-lacZ* fusions in the chromosome, we used the λ phage vector λ RS45 (30) to transfer the fusion by homologous recombination in the *lac recA*⁺ host MC4100 (Table 1). The recombinant λ (*htrP-lacZ*) phage were used to lysogenize MC4100. The lysogens were first picked up as blue colonies and then checked by induction of the λ lysogen and assayed for the phenotype of the released phage.

Assay of β -galactosidase. β -Galactosidase activities were determined by the method of Miller (21). The overnight bacterial cultures grown in either M9 minimal or LB rich medium (19) at 22°C (room temperature) were diluted 1:100 and allowed to grow to an optical density of 0.2 at 600 nm. The cultures were subsequently shifted to either 30 or 37°C for further incubation. Parallel cultures were also grown at 30 and 37°C in the same way. Protein synthesis was inhibited by the addition of chloramphenicol (30 µg/ml), and samples (50 to 100 µl) were added to Z buffer. Sodium dodecyl sulfate (SDS) (0.1%) and chloroform were used to disrupt the cell membrane. Samples were assayed in duplicate, and the data presented here are the average of six independent experiments.

Sequencing. Nucleotide sequence analysis of the 1.6-kb *ClaI-NdeI* chromosomal insert in pSR591 was performed by either subcloning various restriction fragments or using nested sets of exonuclease III-generated subclones. Both strands were sequenced by the dideoxyribonucleotide chain termination reaction technique. The sequencing reactions were done with a Sequenase kit (U.S. Biochemicals, Cleveland, Ohio).

Computer analysis of sequence data. The predicted amino acid sequence of the HtrP protein was compared by the FASTA algorithm of Pearson and Lipman (25) against release 16 of SWISS-PROT and release 63 of GenPept. Potential transmembrane segments were evaluated by the method of Klein et al. (13), using PC Gene software.

Nucleotide sequence accession number. The GenBank accession number for the *htrP* gene is M64472.

RESULTS

A library consisting of at least 5×10^4 mini-Kan::Tn10 insertional events was constructed at 30°C in the wild-type strain B178 by infecting the strain with λ 1105 at a multiplicity of infection of 0.1 phage per bacterium. This library was subsequently screened for isolates with a temperature-sensitive phenotype (Ts⁻), i.e., inability to form colonies at 42°C. One such Ts⁻ isolate, subsequently designated *htrP698::Tn10*, was chosen for the present study. The *htrP* mutation was first shown, by P1 transduction, to confer a Ts⁻ phenotype in various wild-type *E. coli* genetic backgrounds. In all such genetic backgrounds tested, *htrP* mutant bacteria exhibited the following phenotypes: (i) inability to form colonies above 37°C in either rich (LB) or minimal (M9) medium, and (ii) a longer doubling time at 30°C compared with the isogenic wild-type parental strain, resulting in small colonies at 30°C. The fact that both these phenotypes cotransduce 100% (100 of 100 tested) with the kanamycin resistance marker shows that both phenotypes are the consequence of a single mutational event.

Cloning of *htrP* gene. Clones carrying the wild-type *htrP* gene were identified on the basis of complementation of the Ts⁻ phenotype from two libraries of cloned wild-type chromosomal DNA. The use of a library of *E. coli* DNA digested with *PstI* and cloned in the low-copy-number vector pGB2 resulted in the identification of a 6.5-kb *PstI* fragment that complemented both slow growth at 30°C and the Ts⁻ phenotype of *htrP698::Tn10* mutant bacteria. We also used an *E. coli* genomic library cloned in cosmid vector pREG153 (23) to isolate cosmid clones that corrected the Ts⁻ phenotype. All subclones from these cosmids were shown to share the same 6.5-kb *PstI* fragment. Further subcloning experiments resulted in the identification of a 1.6-kb *ClaI-NdeI* minimal DNA fragment that rescued all mutant phenotypes exhibited by the *htrP698::Tn10* mutation (Fig. 1).

Mapping of *htrP* gene. To map the *htrP* gene, we used the ³²P-labeled 6.5-kb *PstI* fragment derived from plasmid pSR589 (*htrP*⁺) to hybridize to the λ *E. coli* DNA library (14). Hybridization was observed with λ clones 507 and 508 (data not shown), which carry the region corresponding to kbp 3220 to 3250 of the *E. coli* physical map.

To further confirm the map position, we first lysogenized *htrP* mutant bacteria with phage λ *imm*⁺ *ind* and then infected them with λ transducing phages 505 to 509. Only recombinant phage λ 507 was found capable of (i) rescuing the Ts⁻ phenotype and (ii) recombining the kanamycin resistance marker from *htrP698::Tn10*. This result places the *htrP* gene close to the 3,240-kb region on the physical map of *E. coli*. By close comparison of restriction maps of *htrP*⁺ subclones derived from cosmids or pSR589 with the restriction map of *E. coli* covering this region (14), the *htrP* gene was further localized within the 3,238- to 3,240-kbp region. The known genes in this area are *tolC* and *cca* (1). These results were confirmed by sequencing (see below). In fact, one of the *PstI* sites of plasmid pSR589 is located within the structural gene of *tolC*.

The *htrP* gene was also mapped genetically by using *tolC::Tn10* as a marker for P1 transduction experiments. Results of such transduction experiments showed that the Tet^r marker from *tolC* and the Kan^r marker from *htrP* are 85% cotransducible. These data are consistent with the rest of the mapping results presented here. The TolC phenotype

FIG. 2. Identification of the *htrP* gene product. (A) Cultures of strains carrying plasmid pBluescript SK⁺ alone or pSR591 with the *htrP* gene cloned under the T7 RNA polymerase promoter. Expression of T7 RNA polymerase was induced by the addition of isopropyl- β -D-thiogalactopyranoside (IPTG; 0.5 mM). Following a 30-min incubation, cells were treated with rifampin (200 μ g/ml) for another 15 min and then labeled with [³⁵S]methionine (50 μ Ci/ml) for 10 min. The proteins were resolved by 12.5% SDS-PAGE. (B) Two-dimensional PAGE of the same [³⁵S]methionine-labeled cell extracts as used in panel A. In the first dimension, 2% pH 3.5 to 10.5 ampholines (Pharmacia) were used. The arrow indicates the position of the HtrP protein.

was monitored by sensitivity to SDS and inability to form colonies on MacConkey agar plates (9).

We also looked into the possibility that the *htrP698::Tn10* mutation lies in the *ribB* gene (2), previously mapped close to *tolC* (1, 2). However, all known insertions in the *ribB* gene exhibit a riboflavin auxotrophy, whereas all of our *htrP* mutant constructs were prototrophic.

Construction of *htrP::* Ω Kan^r insertion mutant. Since we isolated only a single *htrP* allele, *htrP698::Tn10*, which was shown by sequencing to map close to the carboxy terminus of the protein (see below), an additional null allele was constructed as follows. First, the Ω Kan^r cassette was inserted in the *Bgl*II site of plasmid pSR603 (Fig. 1). This DNA was used for linear transformation and recombination of the insertion mutation into the chromosome. The *htrP510::* Ω Kan^r strain thus constructed exhibited the same phenotype as *htrP698::Tn10*. We conclude that the *htrP* gene is dispensable at low temperature but is absolutely required for *E. coli* bacterial viability at temperatures above 37°C.

Identification of HtrP protein. The *htrP* gene product was identified by in vivo expression from its own promoter in the maxicell system (29) as well as in the T7 promoter expression system (32) (Fig. 2). Introduction of the multicopy plasmid pSR590 (*htrP*⁺) or pSR591 (*htrP*⁺) resulted in the overproduction of a 27-kDa protein in both wild-type and *htrP* mutant bacteria. Since a protein of similar size is missing in *htrP* mutant bacteria (Fig. 3), we tentatively concluded that the 27-kDa protein is the product of the *htrP* gene. The *htrP* gene, when exclusively transcribed from the T7 promoter with BL21(DE3) (32) as the host strain, resulted in the production of an identical 27-kDa acidic protein, which migrated at the expected isoelectric charge (24) (pI = 4.55) predicted from the DNA sequence analysis (see below). By the known orientation of the *Nde*I-*Cla*I fragment in plasmid pSR591 or the *Nde*I-*Pst*I fragment in plasmid

FIG. 3. In vivo expression of HtrP protein. Cultures of *htrP698::Tn10* bacteria, carrying either the vector, pBluescript SK⁺, alone or the plasmid pSR591 (*htrP*⁺) were grown at 22°C, shifted to 37°C for 10 min, and then labeled for 10 min with [³⁵S]methionine (50 μ Ci/ml). Equivalent amounts of cells were labeled and extracts were analyzed as in Fig. 2B. The arrow indicates the position of the 28-kDa HtrP protein.

pSR590, with respect to the direction of T7 RNA polymerase transcription, it was found that the *htrP* gene, encoding the 27-kDa protein, is transcribed toward the *Nde*I site, which is counterclockwise, vis-à-vis the conventional *E. coli* genetic map. As can be seen in two-dimensional polyacrylamide gel electrophoresis (PAGE) gels (Fig. 2 and 3), the HtrP protein appears to possess multiple isoelectric forms, suggesting that it is posttranslationally modified.

Sequencing the *htrP* gene. The *htrP* gene was sequenced from DNA subcloned in both the low-copy-number vector pGB2 and Bluescript vectors. (Both strands were sequenced with either derived subclones or exonuclease III-generated overlapping subclones.) Sequence analysis of a 1.6-kb DNA region from the *Cla*I to *Nde*I restriction sites revealed a 756-nucleotide open reading frame (ORF) (Fig. 4). The ORF starts at an ATG, at position 794, and terminates at a TGA, at position 1550. The sequence predicts a 252-amino-acid polypeptide of 27,471 Da, possessing a pI of 4.55. A putative Shine-Dalgarno sequence was located at positions 780 to 783.

The position of the mini-Kan::Tn10 insertion was determined by sequencing the DNA from pSR604. The site of the mini-Kan::Tn10 insertion was found within the deduced *htrP* gene at position 1397 of the sequence (Fig. 4). This places the Tn10 insertion at the position coding for amino acid 202 of the HtrP protein.

```

1   CGATTAAGTAAACTTAAAAATACATTGCAATTATACATTTCAATTGTACTCATTCAATTTTGTGATGGTTT
73  TCTGAATGGGATTACGCGATAAGGTAATAGAAAGCACTATAAATTAGAGGATTAAATTCATAATTTACAGTGC
145 ATTAAGACGAATGTTTACAGCGTTGGGGGAGAGTCTCTGGATCTGGCTGCTTTTGTATCTCAGTACTCGG
217 ATTTCCAGTTCGCTGATTCTTTGTTCAGCAGCGCCAGTTTTCACGAGTCCGTAAACAGGACTTGCCTTTG
    AatII
289 GACGTCGAACCTTTCACGGCTTACAAGGTCGAGCGGTAGCCTGCGCTTGTAGGGTTGGCGGATTTTTTTC
361 TCTTAGGCATTGATTCTGTGAACCTGGCGAGCGATTGCTCAATTTTTTTCGGGTCAATCATGTAGTTTCCC
433 TGTACTGATAGGTGTTGAGTGCCATTGTAGTCCGATAAGGTAAGTCAATAACAGCAATATGTGAAGCTAT
505 GCGTTGCTCCGCTAATCATTAGCGTTATAGTGAATCCGCTTATCTCAGGGCGGGCGAAATTTCCACCGG
577 GCGGTAATCAACTCAGTTGAAAGCCGCGAGCGCTTTGGGTGCGAACTCAAAGGACAGCAGATCCGGTGTA
649 TTCCGGGCGCGACGGTTAGAGTCCGGATGGGAGAGAGTAACGATTCTGCGGGCATGGACCCGCTCACGTTA
    S.D.
721 TTTTGGCTATATGCCGCCACTCCTAAGACTGCCCTGATTCTGGTAACATAATTTAGTGAGGTTTTTTTAC
    AsuII
793 CATGAATCAGACGCTACTTTCTCTTTTGGTACGCCTTTCGAACGTGTTGAAAATGCACTGGCTGCGCTGCG
    M N Q T L L S S F G T P F E R V E N A L A L R 24
865 TGAAGGACGCGGTGTAATGGTGCTTGATGATGAAGACCGTGAAAACGAAGGTGATATGATCTTCCCGCAGA
    E G R G V M V L D D E D R E N E G D M I F P A E 48
937 AACCATGACTGTTGAGCAGATGGCGCTGACCATTCGCCACGGTAGCGGTATTGTTTGCCTGTGCATTACTGA
    T M T V E Q M A L T I R H G S G I G C L C I T E 72
1009 AGATCGCCGTAACAACTCGATCTGCAATGATGGTAGAAAATAACACAGCGCCTATGGCACCGGTTTAC
    D R R K Q L D L P M M V E N N T S A Y G T G F T 96
1081 CGTGACCATTGAAGCAGCTGAAGGTGTGACTACCGGTGTTTCTGCGCTGACCGTATTACGACCGTTCGCAG
    V T I E A A E G V T T G V S A A D R I T T V R S 120
    BglII
1153 CGATTCGATGGCGCAAAACCGTCAGATCTGAATCGTCTGCGCCAGTTTCCCACTTCGCGCTCAGGCAGG
    D S D G A K P S D L N R P G H V F P L R A Q A G 144
1225 TGGTGACTGACGCGTGGCGGTCACTAGCAACTATTGATCTGATGACGCTGGCAGGCTTTAAACCGCG
    G V L T R G G H T E A T I D L M T L A G F K P A 168
1297 TGGTGACTGTGTGAGCTGACTAATGACGATGGCAGGATGGCGGTGACACAGAGTGATTGAGTTTGCCAA
    G V L C E L T N D D G T M A R A P E C I E F A N 192
    ↓
1369 TAAACACAATATGGCGCTCGTGACTATTGAAGACCTGGTGGCATAACCGTCAGCACATGAGCGTAAAGCCAGC
    K H N M A L V T I E D L V A Y R Q H M S V K P A 216
1441 TGAAAACCGTGCTTAATTTACTGCCTTAATCAAGAAACCGAAGTTGTAGCAGGCTTCGGTTTATTTTTC
    E N R C L I Y C L N Q E T E V V A G F G F Y F S 240
1513 CCTGCTATGCAAGATGTTAATTCGCTTCTCTTCTATGAGAAAATTCATTAAATATCAGGCATTCTTTTTC
    L L C K M L I P L L F L 252
1584 ATTATGATCAAAATCATATAATTGTTCCGTTTATTATGGCGGACCATATG

```

FIG. 4. Nucleotide sequence of the *htrP* gene and its flanking regions. The site of the Tn10 insertion at nucleotide position 1397 is indicated by an arrow (↓). The putative Shine-Dalgarno (SD) region is overlined.

Based on the sequence analysis and complementation by the minimal clone carrying 1.6-kb *ClaI-NdeI* DNA fragment, it is concluded that the *htrP* gene is not part of an operon and hence that all the phenotypes associated with *htrP698::Tn10* insertion mutation are due to lack of *htrP* function rather than due to any polar effects on a cotranscribed gene. These results were further confirmed by analysis of the transcripts (see below), which showed that the *htrP* gene encodes a monocistronic message.

Transcriptional regulation of *htrP* gene. Since the *htrP* gene is essential for *E. coli* growth at temperatures above 37°C, we looked at the relative abundance of *htrP*-specific transcripts by Northern analysis. RNA extracted from wild-type cells grown at 30, 42, and 50°C was used. Under all conditions tested, a single transcript of about 850 nucleotides was observed, using the 1.6-kb *ClaI-NdeI* nick-translated DNA as the probe in the Northern analysis. Unlike heat shock genes, the accumulation of *htrP*-specific transcripts was observed to decline with a shift-up in temperature (Fig. 5). We also looked for the presence of *htrP*-specific transcripts in an isogenic *rpoH* null mutant. The *htrP* gene was found to be actively transcribed in the *rpoH* null mutant KY1621 (38). Thus, it is concluded that transcription of the *htrP* gene does not require the presence of the *rpoH* gene product (σ^{32}) and is not subject to any other type of heat shock regulation.

Expression of *htrP* promoter as judged by *lacZ* operon fusions. The putative promoter region of the *htrP* gene was

cloned in the promoter probe operon fusion vector pRS528 (30) as described in Materials and Methods (Fig. 6). The cloning of either the 830-bp *AsuII-ClaI* fragment or the 536-bp *AsuII-AatII* fragment resulted in a *Lac*⁺ phenotype, indicating that an active promoter is present within this

FIG. 5. Northern analysis of *htrP* transcripts. RNA was extracted from isogenic *rpoH*^Δ (KY1621) and *rpoH*⁺ (SR407) strains grown at 30°C (lanes 1 and 4, respectively) or shifted to either 42°C (lanes 2 and 5, respectively) or 50°C (lanes 3 and 6, respectively) for 10 min before extraction. Total RNA (5 μg per lane) was analyzed by the Northern blot technique and probed with 200 ng of the ³²P-labeled 1.6-kb *ClaI-NdeI* DNA fragment that carries the entire *htrP*⁺ gene.

FIG. 6. Construction of plasmid-borne *htrP-lacZ* fusions. (a) The 830-bp *ClaI-AsuII* and 536-bp *AatII-AsuII* fragments were isolated from *htrP*⁺ plasmid pSR590 and cloned into pBluescript SK⁺ (b and c, respectively). These *htrP* promoter-containing inserts were then recloned into the pRS528 operon fusion vector (d and e, respectively). Cross-hatched bars indicate pBluescript SK⁺ vector DNA. Stippled bars indicate pRS528 vector DNA. *AsuII** denotes the hybrid *ClaI-AsuII* site. *SmaI** denotes the hybrid *ClaI-SmaI* or *HincII-SmaI* site.

region. To avoid complications arising from multicopy plasmid effects, the cloned promoter region from pSR950 was transferred onto the λ RS45 transducing phage by homologous recombination. Such recombinant phage were used to introduce a single copy of the *htrP-lacZ* fusion into the chromosome (30). The level of *lacZ* expression was measured by monitoring β -galactosidase activity under different growth conditions. Consistent with the Northern analysis of *htrP* transcription, *lacZ* expression was found to decrease upon a shift-up in temperature. The steady-state levels measured under different growth-phase conditions (Table 2) were three- to fourfold lower at 37°C than those at 22°C. No significant differences in *htrP* promoter-driven *lacZ* expression were observed in the isogenic *htrP*⁺ or *htrP* mutant genetic backgrounds at 22 or 30°C, indicating the absence of self-regulation.

***htrP* cells filament above 37°C.** Introduction of the *htrP* mutation into an otherwise wild-type *E. coli* background resulted in a decrease in viability at temperatures above 37°C (Fig. 7). To account for the decrease in viability at high temperature (37°C and above), we studied *htrP* bacteria, along with isogenic *htrP*⁺ strains, by light microscopy. Cells at different stages of growth in either minimal or LB medium

were analyzed over a wide range of temperatures. No major differences were observed for either colony morphology or generation time at 22°C. The *htrP* bacteria grew as motile rods at 30°C in the early stages of growth, similar to the wild-type parent (Fig. 8). However, when *htrP* bacteria reached the late log phase, approximately 5 to 10% of the

FIG. 7. Growth curves of *htrP*⁺ and *htrP698::Tn10* isogenic bacteria. Exponentially growing cultures at 22°C were diluted 1:10 into prewarmed LB and incubated at 22, 30, 37, or 42°C. Bacterial growth was monitored by measuring CFU per milliliter at various times after the temperature shift by plating serial dilutions. □, 22°C; ♦, 30°C; ■, 37°C; ◇, 42°C.

TABLE 2. β -Galactosidase activity of *lacZ* operon fusions to the *htrP* promoter

Strain and temp (°C)	β -Galactosidase activity (Miller units) with the following optical density at 600 nm of culture at time of sampling:				
	0.2	0.5	0.8	1.5	≥ 2.4
MC4100(λ RS74- <i>plac</i>)					
22	669	706	ND ^a	652	698
30	646	681	ND	716	672
37	781	746	ND	775	769
MC4100(ϕ <i>htrP-lacZ</i>)					
22	2,633	2,714	2,455	2,919	2,973
30	1,514	1,609	1,511	1,787	1,801
37	735	790	712	695	845

^a ND, not determined.

FIG. 8. *htrP* mutants exhibit filamentation at high temperature. Bacterial cell morphology at 22°C of *htrP*⁺ (A) and *htrP* mutant (B) cells. Cultures were grown in LB medium to an optical density of 0.2 (595 nm) and examined under the light microscope. The *htrP* mutant culture was then shifted to 37°C (C) or 42°C (D) for 2 h and examined. The *htrP* mutant cultures were also shifted to 42°C in low-phosphate medium (E) or saline medium (F) for 2 h and examined. Under identical conditions, *htrP*⁺ bacterial cell morphology remained essentially as that at 22°C (see panel A). The bar in panel F represents 5 μ m.

cells were anucleated at 30°C. At 30°C, the *htrP* bacteria grew slowly, with a mean generation time of 75 min compared with 42 min observed for the isogenic *htrP*⁺ strains. In accordance with this, colony size was reduced in *htrP* bacteria compared with the *htrP*⁺ parental strain. When *htrP* cells grown at 22°C were shifted to 37 or 42°C, they either underwent filamentation or exhibited aberrant cell morphology. Filamentation started within 2 h at 42°C following a shift from 22°C. When the cultures were shifted to 37°C, the extent of filamentation varied, probably owing to the comparatively leaky nature of the phenotype at this temperature. We also examined the phenotype in low-phosphate medium (Fig. 8E). In this medium, filamentation was more pronounced at both 37 and 42°C than that observed in LB or minimal medium. The low-phosphate medium was M9 medium without added extra inorganic phosphates but supplemented with 5 mM KCl and Casamino Acids (0.4%). When *htrP* bacteria were incubated in saline solution (50 mM Tris [pH 7.5], 0.84% NaCl) at 37 and 42°C, they lost the normal rod shape and instead appeared round or formed ghostlike heads (Fig. 8F). Under identical conditions, the wild-type bacteria retained both the normal rod shape as well as viability. These results suggest that *htrP* mutant bacteria possess defective cell walls, implicating a direct or indirect effect of the HtrP protein in proper cell wall biosynthesis and/or assembly.

Computer analysis of HtrP protein sequence. Using the method of Kyte and Doolittle (16), we predicted the HtrP protein to be overall hydrophilic in nature. This observation

was verified by isoelectric focusing experiments (Fig. 2 and 3) with [³⁵S]methionine-labeled extracts prepared from cells expressing the HtrP protein by the T7 promoter system. Based on the method of Klein et al. (13), using PC Gene software from Intelligenetics, Inc., the HtrP protein was predicted to be an integral membrane protein, with two transmembrane helices spanning amino acid residues 92 to 109 and 231 to 251, respectively. Since the HtrP protein is not processed and lacks an obvious signal peptide, it is likely that it is either an inner membrane protein or membrane associated. To determine whether HtrP protein is an integral membrane protein or not, we labeled cells with [³⁵S]methionine and fractionated the sonicated whole-cell extracts on sucrose step gradients (11) or by centrifugation at 220,000 $\times$ g for 2 h. When HtrP protein was overproduced by using pSR591 with the *htrP* gene expressed from T7 promoter, most of the HtrP protein was found in the soluble fraction (Fig. 9). This result argues against HtrP protein being an integral membrane protein.

Homology to other proteins. Searches of the protein sequence data bases, using the predicted HtrP sequence, revealed significant homology to the known LuxH protein of *Vibrio harveyi*. These two proteins share an identity of 64.4% over a length of 210 amino acids (Fig. 10). The *luxH* gene is the last gene in the *luxAH* operon (33). The function of the *luxH* gene is not yet known. However, it is known that the expression of *luxH* is not essential for light production (33). Another protein sequence found in the data base, RibA (GenBank X51510), presumably involved in riboflavin bio-

FIG. 9. Fractionation of HtrP protein. Bacterial cultures (25 ml) of strains carrying plasmid pSK⁺ alone or p591 with the *htrP* gene cloned under the T7 RNA polymerase promoter were labeled with [³⁵S]methionine for 10 min as described in the legend to Fig. 2A. The labeled cells were centrifuged at $2,000 \times g$ for 10 min, washed in M9 medium, and resuspended in 0.5 ml of 20% sucrose, followed by the addition of DNase and RNase (10 μ g/ml). Cells were broken by sonication, and unbroken cells were removed by two cycles of centrifugation at $2,000 \times g$ for 10 min. The supernatant was then centrifuged at $220,000 \times g$ for 2 h. The supernatant containing the soluble proteins was precipitated with trichloroacetic acid (10%) and resuspended in 200 μ l of sample buffer. The pellet (membrane fraction) was dissolved in 200 μ l of SDS sample buffer. The total cell extracts (T) and soluble (S) and membrane (M) fractions were then analyzed by 12.5% SDS-PAGE. The arrow indicates the position of the HtrP protein.

synthesis in *Bacillus subtilis*, also shares significant homology (49.4% identity over a length of 198 amino acid residues) with the HtrP protein. However, the RibA protein is much larger than HtrP, consisting of 398 amino acid residues.

DISCUSSION

We identified, cloned, and mapped the *htrP* gene of *E. coli*. The *htrP* gene was identified on the basis of an insertion mutation, *htrP698::Tn10*, which confers a Ts⁻ growth phenotype. Since the *htrP* insertion mutation does not allow growth of *E. coli* cells at temperatures above 37°C, we used this phenotype to clone the wild-type *htrP* gene by complementation. The *htrP* gene maps at 66.3 min, corresponding to the 3,238- to 3,240-kbp region of the physical map of the *E. coli* chromosome. The *htrP* gene is located 4 kbp clockwise from the *tolC* gene, its direction of transcription being counterclockwise relative to the genetic map and in the direction opposite to that of the *tolC* gene (9).

The *htrP* gene was sequenced and found to possess an ORF coding for a protein with a predicted molecular weight of 27,471 and a predicted isoelectric point of 4.55. The evidence that this ORF indeed encodes the HtrP protein is as follows. (i) The predicted and observed sizes (~27,000 Da) (Fig. 2 and 3) of the protein product agree with each other. (ii) There is no other ORF of a similar size within the sequenced area. (iii) The predicted isoelectric charge of HtrP is pH 4.55, in agreement with its observed migration on two-dimensional SDS-polyacrylamide gels (Fig. 3).

HtrP	MNQTLSSFGTFFERVENALALREGRGMVLLDDEDRNEGDMIFPAETMTVEQMAL
LuxH	MSSTSLLEDFGTPVQVRERAIEALKNGLVLLDDEDRNEGDLIFSAQHLTEAQMAL
HtrP	TIRHSGIVCLCITEDRRKQLDLPMMVENNTSAYGTGFTVTIEAAGVTTGVSAADR
LuxH	MIREGSGIVCLCLTEERANWLDLPMMVKDNCNRQTAFTVSEAEKGVTTGVSAKDR
HtrP	ITTVRSDDSD-GAKPSDLNRPFGHVFLRAQAGGVLTTRRGHTTEATIDMTLAGFKPAGV
LuxH	VTTVKATATYFDAQPEDLARPFGHVFLVAKTNGVLARRGHTTEGTDIMYLANLVPSGI
HtrP	LCELTNDGDTMARAPCEIEFANKHNMLVLTIEDLVAYRQHMVSKPAENRCLYICLNQ
LuxH	LCELTNDGDTMAKLPETIEFARRHGMPLTIEDIVYRTGIDLRNEYKSGLVREVSW

FIG. 10. Comparison of amino acid sequences of *V. harveyi* LuxH and *E. coli* HtrP proteins. Alignments were made by the method of Pearson and Lipman (25). Identical residues are indicated by double dots (:); conserved residues are indicated by a single dot (•).

(iv) When the wild-type gene was replaced by either the *htrP698::Tn10* or *htrP510::Ω* Kan mutant allele, the 27,000-Da protein was no longer made. (v) Sequencing of the *htrP698::Tn10* mutation confirmed that the *Tn10* insertion was present in this particular ORF, and (vi) the strong homology between the HtrP protein predicted from this ORF and LuxH protein runs through the length of this ORF, particularly in the first 220 amino acids.

The facts that the minimal *htrP*⁺ clone (1.6-kb *Clai-NdeI*) (i) complements all the phenotypes and (ii) possesses a single ORF and that (iii) a single 850-nucleotide-long *htrP*-specific transcript is found show that the *htrP* gene is not organized as an operon. This implies that all the phenotypes associated with the *htrP698::Tn10* mutation arise because of the absence of *htrP* function and are not due to any polar effects on the expression of some other gene.

Analysis of RNA transcripts shows that (i) transcription of the *htrP* gene is not subject to regulation by Eo³², since the *htrP* gene is transcribed in the *rpoH* null mutant, and (ii) the accumulation of the *htrP*-specific gene transcripts declines upon a shift-up in temperature. These results clearly indicate that the *htrP* gene is not a classical heat shock gene. Using *htrP-lacZ* operon fusions, we further confirmed that the level of transcription from the *htrP* promoter is reduced at high temperatures. However, it is quite interesting that even a shift up from 22°C to 30 or 37°C leads to a progressive decline in the rate of *htrP* promoter-driven *lacZ* expression. Since the reduction was observed at all growth stages, including the stationary phase, the transcription does not appear to be growth rate regulated, at least in the wild-type background used (MC4100). Although transcription from the *htrP* promoter is reduced at 37°C vis-à-vis that observed at 22°C, the transcription rate is still fairly high compared with other known promoters.

The HtrP protein is predicted to be overall hydrophilic in nature, although it has two potential transmembrane segments. Both the lack of a signal peptide sequence and the hydrophilic nature of the protein suggest that the HtrP protein either spans the inner membrane or is membrane associated. However, when the HtrP protein was overproduced from the T7 promoter expression system, it was found that most of the HtrP protein was present in the soluble fraction. It is thus likely that the major fraction of the HtrP protein is cytosolic in nature. Clearly, more experiments are

needed to pinpoint the exact location of the HtrP protein in a system in which the *htrP* gene is present in a single copy. The potential association of the HtrP protein with the *E. coli* inner membrane helps explain the unusual shapes exhibited by *htrP* mutants under certain experimental conditions, i.e., it could be that the lack of HtrP protein causes an inner membrane perturbation, which in turn somehow leads to abnormal cell wall formation. The HtrP protein may be subject to posttranslational modifications, since multiple isoelectric forms were seen in two-dimensional polyacrylamide gels. The nature of these modification(s) is not known at present. It may be interesting to know whether the modifications are temperature regulated, a possibility suggested by the need for HtrP protein only at high temperatures.

The HtrP protein shares a striking homology to the LuxH protein from *V. harveyi*. Since the function of LuxH is not known, the significance of such homology is difficult to interpret. However, another protein, RibA from *B. subtilis*, with which HtrP shares significant homology has been implicated in riboflavin biosynthesis. Our *htrP* mutants exhibit no riboflavin auxotrophy, and their Ts⁻ phenotype is not affected by the exogenous addition of riboflavin. As pointed out earlier, some riboflavin auxotrophs in *E. coli* have been shown to be tightly linked (59 to 96% by P1 transduction) to the *tolC* gene (2). Clearly, more studies are needed to establish a functional and genetic relationship between the HtrP protein and LuxH and to determine whether the HtrP protein is somehow involved in the biosynthetic pathway of riboflavin.

ACKNOWLEDGMENTS

We thank D. Ang for useful discussions and a critical editing of the manuscript and L. Baird for help in preparing figures.

This work was supported by Public Health Service grant AI21029 from the National Institutes of Health.

REFERENCES

- Bachmann, B. J. 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. 54:130-197.
- Bandrin, S. V., P. M. Rabinovich, and A. I. Stepanov. 1984. Three linkage groups of genes for riboflavin biosynthesis. Sov. Genet. 19:1103-1109.
- 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. 104:541-555.
- Churchward, G., D. Belin, and Y. Nagamine. 1984. A pSC101-derived plasmid which shows no sequence homology to other commonly used cloning vectors. Gene 31:165-171.
- Colman, A. 1984. Expression of exogenous DNA in *Xenopus* oocytes, p. 49-69. In B. D. Hames and S. J. Higgins (ed.), Transcription and translation: a practical approach. IRL Press, Washington, D.C.
- 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.
- Fellay, R., J. Frey, and H. Krisch. 1987. Interposon mutagenesis of soil and water bacteria: a family of DNA fragments designed for *in vitro* insertional mutagenesis of Gram-negative bacteria. Gene 52:147-154.
- Georgopoulos, C., and I. Herskowitz. 1971. *Escherichia coli* mutants blocked in lambda DNA synthesis, p. 553-564. In A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Hackett, J., and P. Reeves. 1983. Primary structure of the *tolC* gene that codes for an outer membrane protein of *Escherichia coli* K12. Nucleic Acids Res. 11:6487-6495.
- Ingraham, J. L. 1987. Effect of temperature, pH, water activity, and pressure on growth, p. 1543-1555. In F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
- Ishidate, K., E. S. Creeger, J. Zrike, S. Deb, B. Glauner, T. J. MacAlister, and L. I. Rothfield. 1986. Isolation of differentiated membrane domains from *Escherichia coli* and *Salmonella typhimurium*, including a fraction containing attachment sites between the inner and outer membranes and the murein skeleton of the cell envelope. J. Biol. Chem. 261:428-443.
- Karow, M., and C. Georgopoulos. Submitted for publication.
- Klein, P., M. Kanehisa, and C. DeLisi. 1985. The detection and classification of membrane spanning proteins. Biochim. Biophys. Acta 815:468-476.
- 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.
- Koomey, J. M., R. E. Gill, and S. Falkow. 1982. Genetic and biochemical analysis of gonococcal IgA1 protease: cloning in *Escherichia coli* and construction of mutants of gonococci that fail to produce activity. Proc. Natl. Acad. Sci. USA 79:7881-7885.
- Kyte, J., and R. F. Doolittle. 1982. A simple method for displaying the hydrophobic character of a protein. J. Mol. Biol. 157:105-132.
- Lipinska, B., O. Fayet, L. Baird, and C. Georgopoulos. 1989. Identification, characterization and mapping of the *Escherichia coli htrA* gene, whose product is essential for bacterial growth only at elevated temperatures. J. Bacteriol. 171:1574-1584.
- Lipinska, B., M. Zylicz, and C. Georgopoulos. 1990. The HtrA (DegP) protein, essential for *Escherichia coli* survival at high temperatures, is an endopeptidase. J. Bacteriol. 172:1791-1797.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Marr, A., and J. L. Ingraham. 1962. Effect of temperature on the composition of fatty acids in *Escherichia coli*. J. Bacteriol. 84:1260-1267.
- Miller, J. H. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- 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. American Society for Microbiology, Washington, D.C.
- O'Connor, M., R. F. Gesteland, and J. F. Atkins. 1989. tRNA hopping: enhancement by an expanded anticodon. EMBO J. 8:4315-4323.
- O'Farrell, P. Z., H. M. Goodman, and P. H. O'Farrell. 1977. High resolution two-dimensional electrophoresis of basic as well as acidic proteins. Cell 12:1133-1142.
- Pearson, W. R., and D. J. Lipman. 1988. Improved tools for biological sequence comparison. Proc. Natl. Acad. Sci. USA 85:2444-2448.
- Prentki, P., and H. M. Krisch. 1984. *In vitro* insertional mutagenesis with a selectable DNA fragment. Gene 29:303-311.
- Raina, S., and C. Georgopoulos. 1990. A new *Escherichia coli* heat shock gene, *htrC*, whose product is essential for viability only at high temperatures. J. Bacteriol. 172:3417-3426.
- Raina, S., and C. Georgopoulos. 1991. The *htrM* gene, whose product is essential for *Escherichia coli* viability only at elevated temperatures, is identical to the *rfaD* gene. Nucleic Acids Res. 14:3811-3819.
- Sancar, A., A. M. Hack, and W. D. Rupp. 1979. Simple method for identification of plasmid-coded protein. J. Bacteriol. 137:692-693.
- Simons, R. W., F. Houman, and N. Kleckner. 1987. Improved single and multicopy lac-based cloning vectors for protein and operon fusions. Gene 53:85-96.
- Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel,

- W. Dove, K. J. Jaacks, D. Grossman, J. W. Erickson, and C. A. Gross. 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. *Microbiol. Rev.* **53**:1–24.
32. Studier, F. W., and B. A. Moffatt. 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189**:113–130.
33. Swartzman, E., C. Miyamoto, A. Graham, and E. Meighen. 1990. Delineation of the transcriptional boundaries of the *lux* operon of *Vibrio harveyi* demonstrates the presence of two new *lux* genes. *J. Biol. Chem.* **265**:3513–3517.
34. Tilly, K., N. McKittrick, M. Zylicz, and C. Georgopoulos. 1983. The *dnaK* protein modulates the heat-shock response of *Escherichia coli*. *Cell* **34**:641–646.
35. Way, J. C., M. A. Davis, D. Morisato, D. E. Roberts, and N. Kleckner. 1984. New *Tn10* derivatives for transposon mutagenesis and for construction of *lacZ* operon fusions by transposition. *Gene* **32**:369–379.
36. Way, J. C., and N. Kleckner. 1984. Essential sites at transposon *Tn10* termini. *Proc. Natl. Acad. Sci. USA* **81**:3452–3456.
37. Yamamori, T., and T. Yura. 1982. Genetic control of heat-shock protein synthesis and its bearing on growth and thermal resistance in *Escherichia coli* K12. *Proc. Natl. Acad. Sci. USA* **79**:860–864.
38. Zhou, Y.-N., N. Kusakawa, J. W. Erickson, C. A. Gross, and T. Yura. 1988. Isolation and characterization of *Escherichia coli* mutants that lack the heat shock sigma factor (σ^{32}). *J. Bacteriol.* **170**:3640–3649.