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Identification, Characterization, and Mapping of the *Escherichia coli htrA* Gene, Whose Product Is Essential for Bacterial Growth Only at Elevated Temperatures

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We identified and cloned an *Escherichia coli* gene called *htrA* (high temperature requirement). The *htrA* gene was originally discovered because mini-Tn*10* transposon insertions in it allowed *E. coli* growth at 30°C but prevented growth at elevated temperatures (above 42°C). The *htrA* insertion mutants underwent a block in macromolecular synthesis and eventually lysed at the nonpermissive temperature. The *htrA* gene was located at approximately 3.7 min (between the *fhuA* and *dapD* loci) on the genetic map of *E. coli* and between 180 and 187.5 kilobases on the physical map. It coded for an unstable, 51-kilodalton protein which was processed by removal of an amino-terminal fragment, resulting in a stable, 48-kilodalton protein.

As a typical mesophile, *Escherichia coli* can maintain balanced growth between approximately 10 and 49°C (19). In the range of approximately 21 to 37°C, the rate of *E. coli* growth varies as a simple function of temperature (19). Raising the temperature above 40°C leads to progressively slower growth rates and changes in the cellular content of many proteins (30, 31). The adaptation processes that occur on a shift to high temperature include an increased expression of a set of genes, called heat shock genes, many of which are highly conserved among procaryotic and eucaryotic organisms. These genes are dispersed throughout the chromosome, and their products perform various functions in the cell, most of which are not clearly understood thus far (12, 16, 30, 31, 34). The transcription of the majority of these genes is positively regulated by the product of the *rpoH* gene (previously known also as *htpR* or *hin*; for a review, see references 30 and 31), the σ^{32} subunit of the RNA polymerase holoenzyme (6, 17). Some of these genes are also essential for bacterial growth under normal temperature conditions, for example, the *rpoD* gene, which codes for the σ^{70} subunit of RNA polymerase (30, 31), or *grpE*, which is essential for the replication of bacteriophage λ but which plays an otherwise unknown role in *E. coli* physiology (D. Ang and C. Georgopoulos, submitted for publication). Several heat shock genes, like *lysU*, which codes for an alternate form of lysyl-tRNA synthetase (30, 31, 38), or *lon*, which codes for an ATP-dependent protease (26), are not absolutely essential for bacterial growth. There is also a class of heat shock genes that is conditionally dispensable at low temperatures; i.e., deletion mutants can be constructed at low temperatures but they grow poorly and rapidly accumulate extragenic suppressors (e.g., *dnaK* [7a] and *dnaJ* [S. Sell and C. Georgopoulos, unpublished data]).

Apart from the canonical heat shock genes, the *rpoH* regulatory gene itself is indispensable for cell adaptation to high temperatures (9). It is also known from two-dimensional electrophoresis of total *E. coli* proteins that there are other unidentified proteins that are induced by a temperature shift which do not belong to the *rpoH* regulon (30, 31); these may also be encoded by genes that are essential for bacterial survival at high temperatures.

Generally, the heat shock response, in spite of being a universal physiological phenomenon that occurs in procaryotic and eucaryotic cells, is poorly understood. Better understanding can be gained through the identification of new genes that are essential for adaptation to high temperatures and the characterization of their products. Toward this goal, we constructed libraries of *E. coli* mutants at 30°C that were made by random insertions of a 3-kilobase (kb) mini-Tn*10* transposon (40), the λ *plac*Mu hybrid phage (7), or Tn*5* (4) and screened for mutants that were unable to grow at 42°C. In this way we discovered at least eight unlinked genes whose inactivation by a transposon resulted in the inability of *E. coli* to propagate on L-agar plates at 43°C (S. Raina, O. Cegielska, L. Baird, O. Fayet, B. Lipinska, and C. Georgopoulos, unpublished data). In this report we provide evidence that two of the mutants have mini-Tn*10* insertions in the same gene, which we call *htrA* (high temperature requirement). We cloned and mapped this gene on both the genetic and physical map of *E. coli* and identified its product both in vitro and in vivo. We show that the 51-kilodalton (kDa) *htrA* gene product is unstable and undergoes posttranslational modification by removal of its N-terminal end to give rise to a stable 48-kDa product. The HtrA protein is not a member of the classical heat shock group of proteins whose synthesis is under σ^{32} regulation (12, 30).

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1.

Bacteriophages. Bacteriophages T4D and T4 *sud1* (*dexA*) were received from P. Gauss (13). Bacteriophage λ L47.1 (25) was used as a vector to construct the *E. coli* genomic library and as a source of DNA in Southern blot experiments. Phage λ imm^{λ} *ind* was used to lysogenize the *htrA* mutants prior to complementation tests with the λ2001 transducing phages. Phage λ imm^{434} cI^{+}To was used to lysogenize the *htrA* mutants prior to complementation tests

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TABLE 1. Strains and plasmids used in this study

Strains and plasmids	Genotype or phenotype	Source or reference
E. coli		
B178	W3101 *galE sup*$^+$	14
CG97	*recA56*, Tn*10* 90% cotransducible	Our collection
LN681	F$^-$ *dnaC28* (Tr$^-$) *thyA deoB* (or *deoC*) *gyrA thi supE42*? *trp*::Mu$^+$	11
OFB435	(C600) *thr-1 leu-6 thi-1 lacY1 thyA deoB* (or *deoC*) *fhuA21 htrA22* Tet^r	Made by P1 transduction of the *htrA22* mutation
AQ685	F$^-$ *proA3 lac-3 metD88 metB1 his-29 trpA9605 thyA deoB* (or *deoC*) *rpoB argH*	T. Kogoma
Hfr3000 YA139	Hfr *panB6 relA1 spoT1 thi-1*	B. Bachmann
AT986	Hfr *dapD8 relA1 spoT1 thi-1*	B. Bachmann
71.18	Δ*lac-pro supD*/F' *lacI*^{q} *lacZ*ΔM15 *pro*+	27; J. Messing
HR42	*optA*$^+$	13; P. Gauss
HR44	*optA1*	13; P. Gauss
Plasmids		
pEMBL8$^+$	Ap^r vector plasmid	10
pLN47	pBR322 derivative containing 12.5 kb of phage Mu DNA	11
pBS28	pBR325 with a 5-kb *Pst*I insert from the region around coordinate 1,659 kb on the physical map of the *E. coli* chromosome	J. P. Bouche
pREG153	Ap^r cosmid	22; D. Low

with the λ L47.1 transducing phages. Strains λ *imm*^{434} *dnaJ*$^+$, λ *imm*^{434} *dnaK*$^+$, λ *imm*^{434} *grpE*$^+$, and λ *imm*^{434} *groES*$^+$ *groEL*$^+$ transducing phages and λ *c*I857*S7* were from our collection. Phage λ 1098, which contained the mini-Tn*10* transposon, was obtained from J. C. Way (40). Phage P1L4, which was used in transduction studies, was obtained from Lucien Caro.

***E. coli* genomic library in λ L47.1 vector.** An *E. coli* DNA library, which was prepared by partial digestion with *Sau*3A restriction nuclease and which was cloned in λ vector L47.1 (*imm*^{434} *c*I), was constructed by Sam Cartinhour.

***E. coli* genomic library in λ 2001 vector.** The *Sau*3A-generated library was obtained from Y. Kohara (21).

Media. M9 minimal medium was prepared as described by Maniatis et al. (25) and was supplemented with glucose (0.3%), thiamine (2 μg/ml), $MgSO_4$ (1 mM), $MgCl_2$ (3 mM), $CaCl_2$ (0.1 mM), and $FeCl_3$ (0.3 μM). M9 high-sulfur medium, which was supplemented with a mixture of defined amino acids and which was used for labeling with [^{35}S]methionine, has been described previously (15). L- and T-broth have been described previously (2). When necessary, the media were supplemented with ampicillin (40 μg/ml) or tetracycline (15 μg/ml).

Enzymes. Restriction enzymes were purchased from New England BioLabs, Inc. (Beverly, Mass.), and Bethesda Research Laboratories, Inc., Gaithersburg, Md.). T4 DNA ligase was from Bethesda Research Laboratories, Inc. DNA polymerase I and DNase I, which were used for nick translation, were from Boehringer Mannheim Biochemicals (Indianapolis, Ind.).

Genetic techniques. In order to construct an *E. coli* mini-Tn*10* library of mutants, strain B178 bacteria were mutagenized by the random insertion of a 3-kb derivative of a Tn*10* transposon (mini-Tn*10*) into chromosomal DNA. For that purpose, λ 1098 carrying the mini-Tn*10* (element 8) was used essentially by the method described by Way et al. (40). A library consisting of about 50,000 independent insertion events was obtained.

In order to isolate λ *htrA*$^+$ transducing phages, *E. coli htrA* mutant cells were lysogenized with λ *imm*^{434} *c*I$^+$To phage (when phages from the λ L47.1 library were tested) or with λ *imm*$^{\lambda}$ *ind* phage (when phages from the λ 2001 library were tested). The lysogens were grown overnight at 30°C in L-broth, and 100 μl of this culture was mixed with about 10^8 PFU of the λ transducing phage library. After 30 min of incubation at room temperature, cells were plated onto L-agar plates and incubated at 30 and 42°C. Abundant growth of normal-looking colonies at 42°C indicated potential lysogenization by λ *htrA*$^+$ transducing phage.

For complementation tests, the plasmid to be tested was transformed into *E. coli htrA22* or *E. coli htrA63*, and appropriate fractions were plated onto L-agar plates that were supplemented with ampicillin at 30 and 42°C. Control transformations with the vector plasmid alone were carried out in parallel. We assumed that suppression was positive when the number of transformants obtained at 42°C was at least 80% of that obtained at 30°C and when the morphology of the colonies was similar to that of the wild type.

For P1 transductions, phage P1L4 was grown and used in transduction experiments essentially as described by Miller (29).

Biochemical techniques. *E. coli* CG97 (*recA56* Tet^r) was transformed with the appropriate plasmid, and the transformant was used to detect expression of the plasmid-encoded proteins following UV irradiation, essentially as described by Sancar et al. (36). The cells were labeled in high-sulfur M9 medium with [^{35}S]methionine (~1,000 Ci/mmol; ICN Radiochemicals, Irvine, Calif.) at a final concentration of 175 μCi/ml. The labeled proteins were analyzed by one- or two-dimensional electrophoresis (see below).

The procaryotic transcription-translation system was purchased from Amersham Corp. (Arlington Heights, Ill.) and was used according to the suggestions of the manufacturer.

The procedure used for isoelectric nonequilibrium focusing in the first dimension was that of O'Farrell et al. (32) for basic proteins, and sodium dodecyl sulfate-polyacrylamide (12.5%) gel electrophoresis in the second dimension was performed as described previously (2, 15).

The method used for peptide mapping was essentially that described by Cleveland et al. (8), in which we used α-chymotrypsin and *Staphylococcus aureus* V8 protease, which were purchased from Sigma Chemical Co. (St. Louis, Mo.) and Miles Laboratories, Inc. (Elkhart, Ind.), respectively.

Preparation of membrane and cytoplasmic fractions. Total cell membranes were prepared as described by Billmire and Duckworth (5), except that the cells were disrupted by sonication instead of with a French press. Cytoplasmic proteins were precipitated from the supernatant fractions by adding trichloroacetic acid to 10%, and they were subsequently washed with acetone. The outer and inner membranes were separated by extraction with Triton X-100 (5).

The procedures used for plasmid and bacteriophage DNA preparation, digestion with restriction enzymes, ligation,

and DNA electrophoresis were essentially those described by Maniatis et al. (25).

The method used for DNA transformations was that described by Hanahan (18).

For Southern blotting experiments, following electrophoresis on 0.7% agarose, DNA fragments were transferred to nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, N.H.) as described by Maniatis et al. (25) and hybridized to the appropriate probe essentially as described by Wahl et al. (39). The probes were bacteriophage or plasmid DNAs and were labeled by nick translation (25) with [^{32}P]dCTP (3,000 Ci/mmol; Dupont, NEN Research Products, Boston, Mass.).

The mapping procedure based on the determination of the replication time of defined DNA segments in synchronized cell culture has been described previously (11). The mapping procedure based on hybridization experiments of cloned DNA segments to the *E. coli* genomic library in phage λ was as follows. Each of the 476 λ transducing phages from the λ 2001 library (21) was spotted onto a square L-plate seeded with a lawn of *E. coli* B178. A grid with 22 rows and 22 columns of 3.5-mm^2 was placed under the plate as a guide. Approximately 0.25 μl of each phage was spotted with a pipette (Pipetman) by just touching the tip to the lawn. The entire library was spotted onto one plate by this method. The plate was incubated overnight at 37°C to allow plaque formation. The plaques were then lifted onto a nylon membrane (Hybond N; Amersham) according to the directions of the manufacturer. The 1.3-kb *Pst*I-*Pst*I fragment from pBL13, which contained most of the *htrA* gene, was purified from agarose using Geneclean (produced by Bio101) by using the protocol of the manufacturer. The fragment was radiolabeled by nick translation and used as a probe in hybridization to the membrane carrying the λ library, as described above for Southern blotting experiments.

Molecular weight standards. Protein molecular weight standards were purchased from Pharmacia Fine Chemicals (Piscataway, N.J.). Prestained protein molecular weight standards and DNA size standards (λ DNA digested with *Bst*EII enzyme) were purchased from Bethesda Research Laboratories and New England BioLabs, respectively.

RESULTS

Isolation of *E. coli* mutants in the *htrA* gene. A library of *E. coli* mutants carrying insertions of a 3-kb Tn*10* transposon derivative (mini-Tn*10*) in various nonessential regions of the chromosome was constructed as described above. Tetracycline-resistant (Tet^r) mutants that grew at 30°C but that were unable to grow at 42°C on a rich medium (L-agar plates) were isolated from the library and subsequently tested by P1 transduction. Mutants which showed 100% cotransduction of the temperature-sensitive (Tr$^-$) and Tet^r phenotypes were investigated further. In order to determine whether a mutation was located in any well-known heat shock genes, we did complementation tests using the following λ transducing phages: λ *dnaK*$^+$, λ *dnaJ*$^+$, λ *grpE*$^+$, and λ *groES*$^+$ *groEL*$^+$. We found at least eight complementation groups of mutants which could not be suppressed by any of the transducing phages listed above and assumed that the mutations occurred in other loci whose products were essential for bacterial survival only at high temperatures. One complementation group consisted of two independent isolates, *E. coli htrA22* and *E. coli htrA63*. These mutations could not be separated by P1 transduction but could be complemented by the same λ transducing phages (see below). Further analysis showed that these insertions were located in the same 1.3-kb *Pst*I-*Pst*I fragment of the *E. coli* chromosome and affected the synthesis of the same protein (see below).

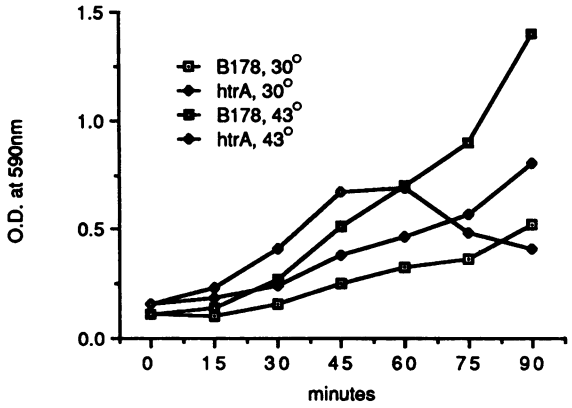

FIG. 1. Growth curves of *htrA*$^+$ and *htrA63* isogenic bacteria. The L-broth cultures were incubated either continuously at 30°C or shifted to 43°C at time zero. OD, Optical density.

Mutations in the *htrA* gene resulted in bacterial lethality at temperatures above 42°C when the bacteria were grown on rich medium (L, T, or M9 medium supplemented with 0.5 to 1.0% Casamino Acids [Difco Laboratories, Detroit, Mich.]) as well as on M9 medium supplemented with 0.3% glucose. Surprisingly, we found that the addition to the minimal medium of serine or leucine at a concentration of 2.5 mM partially suppressed the Tr$^-$ phenotype, allowing the formation of small colonies. This was an unexpected result, since both amino acids were also present in the rich medium. Evidently, their protective function is insufficient when cells grow rapidly, when other substances that are present in the rich media interfere with this function, or both. We did not pursue this finding further.

We determined the rates of synthesis for DNA, RNA, and protein in the *htrA* mutants at both the permissive (30°C) and nonpermissive (43.5°C) temperatures. Following a shift to the nonpermissive temperature, the *htrA* mutants exhibited lower rates of DNA, RNA, and protein synthesis; this was followed by cell lysis. Generally, the richer the medium (and, hence, the faster the growth), the more rapidly this process occurred. The growth pattern of isogenic *htrA*$^+$ and *htrA63* bacteria growing in L-broth at 30 and 43°C is shown in Fig. 1. As can be seen, the *htrA63* culture began to lyse at 60 min following the shift to 43°C. The colony-forming ability of *htrA63* bacteria began to decline coincident with the decline in optical density (data not shown).

Isolation of λ *htrA*$^+$ transducing phages. The *E. coli* genomic library cloned in the λL47.1 vector was used to isolate λ *htrA*$^+$ transducing phages. Five transducing phage strains carrying the bacterial segment which included the *htrA* gene were selected on the basis of their ability to suppress the Tr$^-$ phenotype of an *E. coli htrA63* (λ *imm*^{434} *c*I$^+$To) lysogen. The λ *htrA*$^+$-4 and -5 transducing phages were arbitrarily chosen for further characterization. Both suppressed the Tr$^-$ phenotype of the *htrA22* mutation equally well.

In order to confirm that the isolated λ transducing phages carried the *htrA* gene (and not an extragenic suppressor of the gene), we labeled the DNA of these phages by nick translation and used it to probe Southern blots of chromosomal DNA derived from either *htrA*$^+$ or *htrA* bacteria and

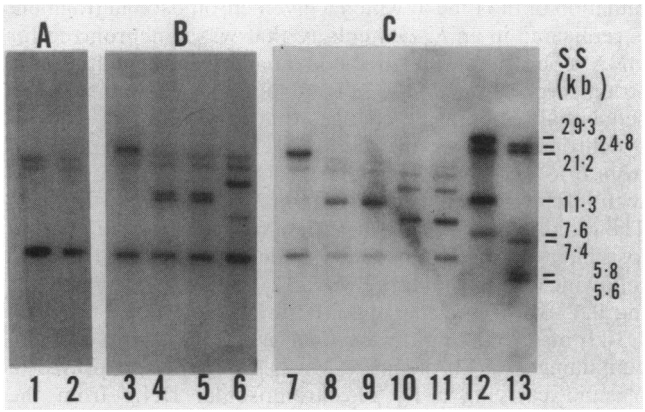

FIG. 2. Testing of the λ *htrA*$^+$ transducing phages for the presence of the *htrA*$^+$ gene. DNA extracted from strain B178 (lanes 1 to 3 and 7), *htrA22* (lanes 4, 5, 8, and 9), and *htrA63* (lanes 6, 10, and 11) by the method of Silhavy et al. (37) was digested with *Eco*RI, electrophoresed on 0.7% agarose, transferred to nitrocellose, and probed with nick-translated DNA of the λ L47.1 vector (A) and λ *htrA*$^+$-5 (B) or λ *htrA*$^+$-4 (C) transducing phages. Lanes 12 and 13 contain size standards (SS), which were made up of λ L47.1 DNA (lane 12) and λ *c*I857*S*7 DNA (lane 13) digested with *Eco*RI and probed with DNA from λ *htrA*$^+$-5 phage.

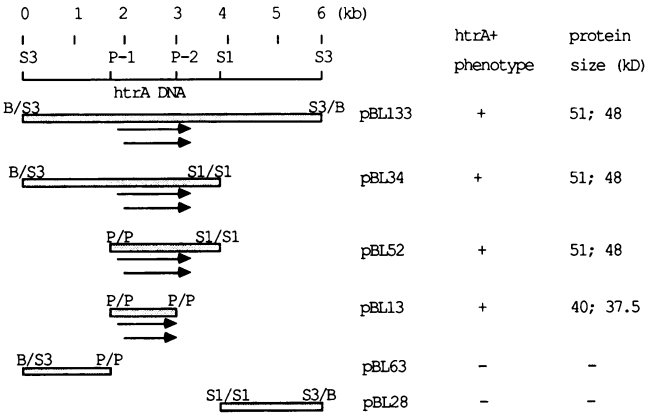

FIG. 3. Subcloning of the *htrA*$^+$ gene. The sizes and relevant restriction sites of the inserts carried by plasmids derived from pBL133 are shown. Plasmid pEMBL8$^+$ served as a vector in all cases. The plasmids were tested for suppression of the HtrA$^-$ phenotype of both *htrA22* and *htrA63* mutants. The arrows show the approximate coding regions for the HtrA polypeptides (see text). Abbreviations: B, *Bam*HI; P, *Pst*I; S1, *Sal*I; S3, *Sau*3A.

digested with *Eco*RI (Fig. 2). In the *htrA*$^+$ DNA pattern, a fragment of about 18 kb was found which hybridized specifically to both λ *htrA*$^+$ phage DNAs (Fig. 2, lanes 3 and 7). This fragment was not seen in DNA of either of the *htrA* mutants. This was expected because they should each carry a 3-kb mini-Tn*10* transposon containing a single *Eco*RI site (40). In the *htrA63* mutant two fragments of about 12.3 and 8.5 kb (Fig. 2, lanes 6, 10 and 11) could be seen, while in the *htrA22* mutant two fragments of about 10.8 and 10.1 kb (Fig. 2, lanes 4, 5, 8, and 9) hybridized to the λ *htrA*$^+$ DNA. The intensity of hybridization depended on which transducing phage was used as a probe (compare lanes 6 and 11 in Fig. 2). In the case of *htrA22* DNA probed with λ *htrA*$^+$-4, the fragments sometimes were not resolved well, because the larger faint one became overshadowed by the heavy lower one (Fig. 2, lanes 8 and 9). In theory, the sum of the molecular weights of the fragments obtained in the mutants should equal the sum of the molecular weights of the wild-type fragment and the mini-Tn*10* insertion (18 kb + 3 kb = 21 kb). In the case of DNA from *htrA63*, the sum was 12.3 kb + 8.5 kb = 20.8 kb, and in the case of DNA from *htrA22* the sum was 10.8 kb + 10.1 kb = 20.9 kb. These numbers correspond reasonably well with the expected size of 21 kb. These results show that both λ *htrA*$^+$-4 and -5 transducing phages carry the *htrA* region of the *E. coli* chromosome. The different sizes of the fragments obtained in the case of DNA from the *htrA63* and *htrA22* mutants indicate that the mini-Tn*10* insertions in these strains are located at different positions in the *htrA* gene (the size differences were too large to be accounted for by different orientations of the transposon inserted in the same position).

Construction of *htrA*$^+$ plasmids. The DNA isolated from the λ *htrA*$^+$-4 and -5 transducing phages was partially digested with *Sau*3A nuclease and ligated into the *Bam*HI site of the pEMBL8$^+$ vector. The ligation mixtures were transformed into *E. coli* 71.18 and plated in the presence of ampicillin, isopropyl-β-D-thiogalactopyranoside, and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) (25). White colonies, which presumably carried recombinant plasmids, were pooled. About 200 colonies were derived from λ *htrA*$^+$-4, and about 500 colonies were derived from *htrA*$^+$-5. The pooled colonies were grown overnight in 250 ml of L-broth supplemented with ampicillin, and plasmid libraries were prepared. The plasmid libraries were used to transform *htrA63* mutant bacteria. Sixty-one transformants that were able to grow at 42°C were selected. All these temperature-resistant transformants were derived from the λ *htrA*$^+$-5 DNA library. We do not know the reason why we did not obtain λ *htrA*$^+$ plasmids from the λ *htrA*$^+$-4 DNA library. All the *htrA*$^+$ plasmids suppressed the Tr$^-$ phenotype of both *htrA22* and *htrA63* mutants. Ten of the *htrA*$^+$ plasmids were analyzed further by digestion with *Eco*RI, *Hin*dIII, and *Pst*I restriction enzymes (data not shown). As the restriction patterns were very similar, only one of these plasmids, pBL133, which carried a 6-kb insert, was chosen for use in further experiments. In order to find the smallest DNA fragment that was able to suppress the HtrA$^-$ phenotype, a series of plasmids was created by subcloning parts of the plasmid pBL133 insert into the pEMBL8$^+$ vector (Fig. 3). Results identified plasmid pBL13, which carried a 1.3-kb *Pst*I-*Pst*I fragment, as being able to correct the Tr$^-$ phenotype of *htrA* mutants. To confirm that both plasmids pBL133 and pBL13 carried the *htrA*$^+$ locus, we performed a Southern blotting experiment (Fig. 4) and showed that the 1.3-kb *Pst*I-*Pst*I fragment was present in DNA from *htrA*$^+$ bacteria (Fig. 4, lanes 7 and 10) but was absent in DNA from *htrA* mutants (Fig. 4, lanes 8, 9, 11, and 12). Since the 3-kb mini-Tn*10* transposon does not have a *Pst*I recognition site (40), we expected the mutated strains to possess an extra 4.3-kb DNA fragment that hybridized to the DNA of the plasmids that were tested. Such a fragment can be seen in Fig. 4 (lanes 8, 9, 11, and 12). This result demonstrates that we indeed cloned the *htrA*$^+$ region and, furthermore, that both mini-Tn*10* insertions are located inside the 1.3-kb *Pst*I-*Pst*I fragment.

We did not attempt to obtain clones that carried inserts smaller than the 1.3-kb *Pst*I fragment, because further analysis of the *htrA* mutations showed that insertion of the mini-Tn*10* transposon 319 base pairs from the left *Pst*I site or 210 base pairs from the right *Pst*I site (Fig. 3, restriction

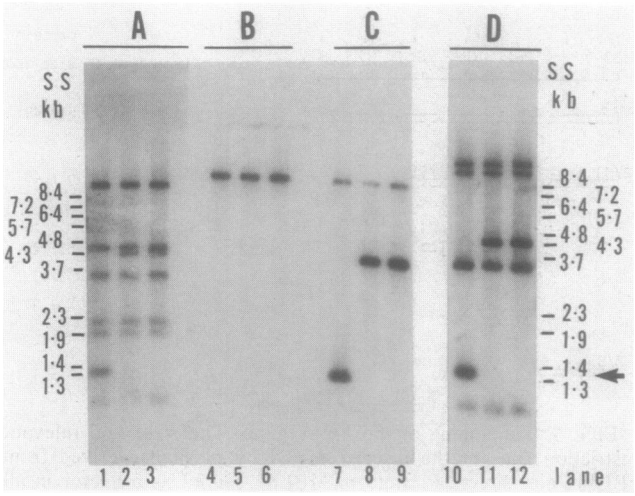

FIG. 4. Testing plasmids pBL13 and pBL133 for the presence of the *htrA* gene. DNA extracted from B178 (lanes 1, 4, 7, and 10), *htrA22* (lanes 2, 5, 8, and 11), and *htrA63* (lanes 3, 6, 9, and 12) was digested with *Pst*I, electrophoresed on 0.7% agarose, transferred to nitrocellulose, and probed with labeled DNA from λ *htrA*$^+$-5 transducing phage (A), pEMBL8$^+$ vector (B), plasmid pBL13 (C), and plasmid pBL133 (D). The arrow points to the 1.3-kb *Pst*I-*Pst*I fragment that was present in B178 DNA and that was absent from *htrA* DNA. A to C and D were separate gels. Each gel contained size standards (SS): λ cI857*S7* DNA digested with *Bst*EII and probed with λ DNA.

map) abolishes the ability of pBL13 to suppress the Tr$^-$ phenotype (24).

Mapping of the *htrA* gene. In order to estimate the position of the *htrA* gene, we used a technique based on the determination of the time at which a given chromosomal fragment is replicated in an *E. coli* culture that was synchronized for DNA replication (11). An exponentially growing culture of a strain carrying the *dnaC28* (Tr$^-$) mutation was submitted to sequential temperature changes that resulted in an efficient synchronization, for one round, of chromosomal replication from *oriC* (11). Newly synthesized DNA was pulse-labeled with [^{3}H]thymidine at regular intervals (see Fig. 5 legend). The radioactive samples were then hybridized to nitrocellulose filters that were loaded with denatured DNA from the following four plasmids: pEMBL8$^+$ (vector plasmid containing 0.45 kb of the *lac* region), pBL13 (pEMBL8$^+$ with a 1.3-kb insert making up the *htrA* gene), pLN47 (a plasmid containing 12.5 kb of phage Mu DNA [11]), and pBS28 (a plasmid carrying 5 kb of chromosomal DNA from the 34.5-min region). These last two plasmids served as reference markers for monitoring the kinetics of replication of the clockwise-replicated half of the chromosome (pLN47) and of the counterclockwise-replicated half of the chromosome (pBS28).

The hybridization peaks obtained for each plasmid (data not shown) were integrated as described previously (11) in order to estimate by extrapolation the replication half-time (i.e., the time required to replicate half of the sequences homologous to a given plasmid probe) of each of the four markers (Fig. 5). Since the *oriC*-pLN47 and *oriC*-pBS28 insert distances were known, the average replication velocity along each replication arm was easily calculated (see Fig. 5 legend); they were 70.89 kb min^{-1} (clockwise arm) and 79.69 kb min^{-1} (counterclockwise arm). In the case of plasmid pBL13, its replication half-time of 14.2 min indicated that its insert was located either 1,007 or 1,132 kb from *oriC* (Fig. 5). The *htrA*$^+$ gene must therefore be located at

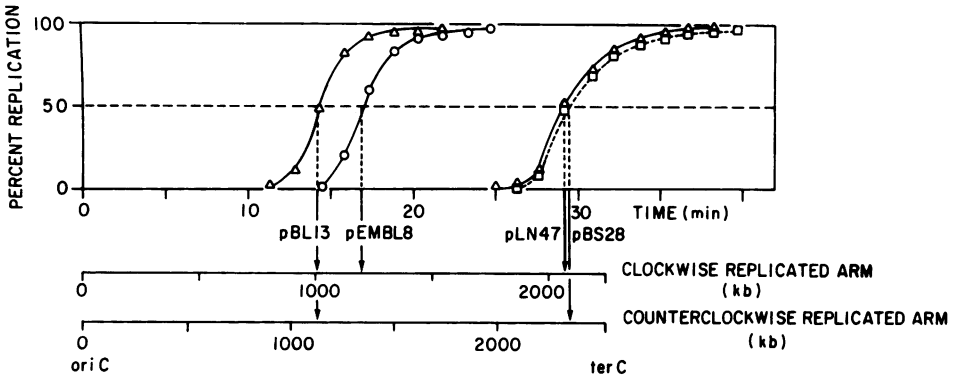

FIG. 5. Mapping of the *htrA*$^+$ gene by DNA hybridization. A culture of strain LN681 was synchronized for one round of DNA replication (11). Samples were removed every 90 s and pulse-labeled with [^{3}H]thymidine for 90 s. The first 32 samples were hybridized as described by Fayet and Prere (11) to filters that were loaded with plasmids pBL13, pEMBL8$^+$, pLN47, and pBS28 (see Table 1 for plasmid descriptions); and the radioactivity that was retained on each filter was measured. The sigmoidal curves were obtained by integration of the hybridization peak that was observed for each plasmid probe (11). Points for which no radioactivity was found were omitted. The pBL13 data had to be corrected because this plasmid and pEMBL8$^+$ contained the same 0.45-kb fragment from the *lac* region. In order to obtain the *htrA* signal alone, the counts per minute bound to the pEMBL8$^+$ filters were corrected for the difference in hybridization yield (i.e., multiplied by 0.47) and then subtracted from the counts per minute bound to the corresponding pBL13 filters. The total radioactivity retained (100% replication) was 320 cpm for pBL13, 175 cpm for pEMBL8$^+$, 2,500 cpm for pLN47, and 1,500 cpm for pBS28. The replication half-times of the four probes were 4.2 min (pBL13), 16.8 min (pEMBL8$^+$), 29.2 min (pLN47), and 29.4 min (pBS28). Two physical distance scales are represented under the abscissa. They allow the correlation of any replication half-time value to a distance (in kilobases) from the origin of replication (*oriC*) on one or the other replication arm. Their establishment was based on the following calculations. By including the 36 kb of the Mu prophage, the total length of the chromosome was assumed to be 4,756 kb (11, 21). The *oriC* region was positioned at 4,038 kb, since our strain LN681 did not have the *rrnE-rrnD* inversion of strain W3110 (21). The center of the Mu prophage (inserted in the *trp* operon and hybridizing with the pLN47 probe) was placed at coordinate 1,352 kb, and the insert of pBS28 was placed at coordinate 1,695 kb. The average replication velocity on the clockwise replicated arm, which was obtained by dividing the *oriC*-Mu prophage distance (2,070 kb) by the replication half-time of pLN47 (29.2 min), was 70.89 kb min^{-1}. For the counterclockwise replicated arm, the velocity was 79.69 kb min^{-1} since the *oriC*-pBS28 distance was 2,343 kb and the pBS28 replication half-time was 29.4 min. The average termination point (*terC*) was therefore located at coordinate 1,521 kb on the 4,756-kb map. Symbols: △, pBL13; ○, pEMBL8$^+$; △, pLN47; □, pBS28.

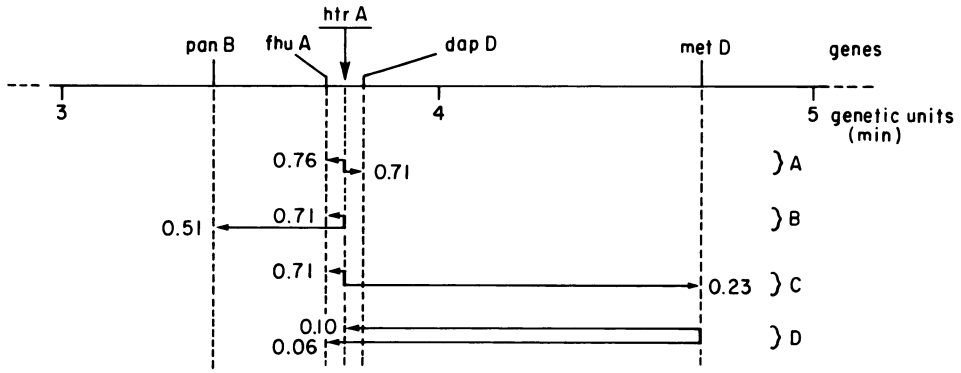

FIG. 6. Transductional analysis of the position of the *htrA22* allele relative to the *panB*, *fhuA*, *dapD*, and *metD* genes. A P1 stock prepared on strain OFB435 (*fhuA21 htrA22*; see Table 1) was used for all crosses. The recipient strains were AT986 (*dapD*) (A), Hfr3000YA139 (*panB6*) (B), and AQ685 (*metD88*) (C and D). The selected marker is indicated by a vertical bar. Selection was for the tetracycline resistance conferred by *htrA22* (A, B, and C), and the *metD*$^+$ transductants were selected (D). In all crosses about 300 transductants were tested for the two other markers (e.g., for *dapD*$^+$ and *fhuA21* [A]) that could be cotransferred with the selected gene. The unselected markers are indicated by arrows, and the cotransduction frequencies are given.

about coordinates 288 or 2,906 kb on the physical map established by Kohara et al. (21), which corresponds to approximately 6.3 or 59.4 min on the genetic map (21).

Phage P1 transductions were carried out in order to ascertain the position of the *htrA*$^+$ gene relative to neighboring genes. In three-factor crosses we measured the cotransduction of *htrA22* (Tet^r) with mutant alleles in the *panB*, *fhuA*, *dapD*, and *metD* genes (3). The results (summarized in Fig. 6) indicate that the mini-Tn*10* insertion generating the *htrA22* mutation is located between the *fhuA* and *dapD* genes, which are positioned at 3.7 and 3.8 min, respectively, on the genetic map (3). These positions correspond to coordinates 177 and 182 kb on the physical map (21). Since a mutation in the *optA1* gene (35) has also been located between the *fhuA* and *dapD* markers, we transduced an *optA1*-carrying strain to tetracycline resistance and found that the OptA$^+$ phenotype cotransduces with *htrA22* at a frequency of 0.92; i.e., the distance between the *optA* and *htrA* genes is approximately 0.06 min, or approximately 3 kb. The presence of the *optA1* mutation was tested by determining the resistance of the strain to phage T4 *dexA* (13) and sensitivity to phage T4$^+$.

Location of the *htrA* gene on the *E. coli* physical map. Since the publication of the physical map of the whole *E. coli* chromosome by Kohara et al. (21), it has become possible to correlate the genetic and physical mapping results. We used this λ library of Kohara et al. (21) to localize precisely the position of the *htrA*$^+$ gene on the physical map. We performed complementation tests with λ 2001 transducing phages 4E11, 17C11, 11C5, 15A7, 9H2, 23G6, 4E4, 12D5, and E2B8; this covered the region between 3 and 5 min on the genetic map (Fig. 7A). We found that two clones, λ 9H2 (117) and λ 4E4 (118), complemented the Tr$^-$ phenotype of *E. coli htrA* mutants. Since the 15A7 phage did not comple-

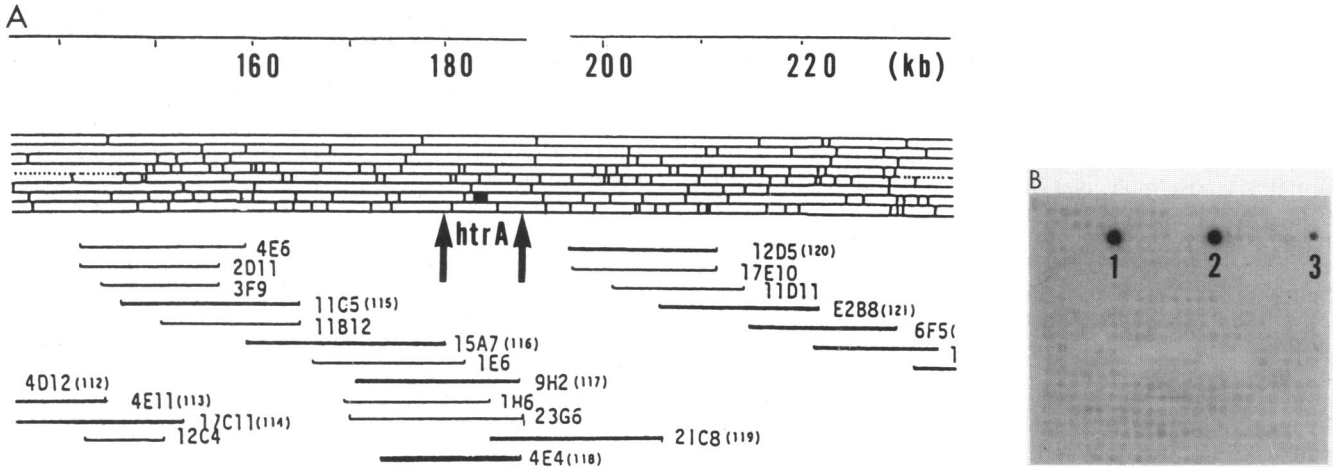

FIG. 7. Localization of the *htrA*$^+$ gene on the *E. coli* physical map. (A) An *E. coli* restriction map of the *htrA*-containing region based on a revised version (Y. Kohara, personal communication) of the published map (21). Horizontal bars indicate the positions of the restriction sites (from top to bottom, respectively) for the following restriction endonucleases: *Bam*HI, *Hin*dIII, *Eco*RI, *Eco*RV, *Bgl*I, *Kpn*I, *Pst*I, and *Pvu*II. Horizontal lines below the map indicate the extent of the chromosomal fragments carried by λ transducing phages. Phage designations are given on the right side of each fragment. The arrows point to the region between 180 and 187.5 kb in which the *htrA*$^+$ gene was mapped. A *Pst*I-*Pst*I fragment of approximately 1.3 kb, which most likely corresponded to the 1.3-kb *Pst*I-*Pst*I fragment of the *htrA* gene, is indicated in black. (B) Plaque hybridization test. The entire λ genomic library was probed with ^{32}P-labeled *htrA*$^+$ DNA (the 1.3-kb *Pst*I-*Pst*I fragment). The λ clones 9H2, 4E4, and 23G6 are marked 1, 2, and 3, respectively.

ment the *htrA* mutations, we concluded that the *htrA* gene is located between 180 and 187.5 kb on the *E. coli* physical map. Surprisingly, the 23G6 phage (which is almost identical to the 9H2 phage) gave negative complementation results (see below).

To confirm the mapping results, we carried out a plaque hybridization experiment (as described above), in which we probed the DNA of the λ library of Kohara et al. (21) with a radiolabeled, 1.3-kb *PstI-PstI* fragment that carried most of the *htrA* gene. Positive hybridization results were obtained with phages 9H2, 4E4, and 23G6 (Fig. 7B). This result further supports the previous conclusion that the *htrA*$^+$ gene is located between 180 and 187.5 kb on the physical map. Comparison of the *E. coli* restriction map of this area (Fig. 7A) with the restriction map of the *htrA*$^+$ gene (Fig. 3) showed that there is a short *PstI* fragment which most likely corresponds to the 1.3-kb *PstI-PstI* region of the *htrA* gene. We do not know why the 23G6 phage hybridized but did not complement the *htrA* mutations. Explanations include the possibility that (i) phage 23G6 was improperly characterized or (ii) it contains a deletion of the *htrA* gene.

Expression of the *htrA* gene in vivo. In order to identify the *htrA*$^+$ gene product, we used both in vivo (maxicells) and in vitro (coupled transcription-translation system) techniques. The maxicell technique (36) was used to identify the *htrA*$^+$ gene product in vivo. We found that in maxicells harboring plasmid pBL133 (Fig. 8A, lane 2) and pBL52 (data not shown), two prominent proteins of approximately 51 and 48 kDa were synthesized, with the smaller one being more abundant. The 48-kDa protein was overproduced so abundantly that it could be visualized by simply staining the protein gels with Coomassie brilliant blue (data not shown).

Restriction analysis of the pBL52 plasmid (Fig. 3) showed that there is no sufficient coding capacity for both 51- and 48-kDa proteins. To test whether these two peptides represent a precursor product of the same gene, we carried out a pulse-chase experiment in the maxicell system. This showed that the 51-kDa protein is very unstable and is being processed to a 48-kDa stable protein (Fig. 8B). However, our results do not exclude the possibility that some of the 48-kDa protein could be the product of independent initiation of translation that occurs efficiently in vivo but not in vitro (see below). The 48-kDa peptide is not a result of independent transcription initiation, because the cells synthesize only one transcript from the *htrA* region (24).

Maxicells harboring plasmid pBL13, the smallest plasmid suppressing the HtrA$^-$ phenotype, synthesize a pair of proteins whose molecular sizes are about 40 and 37.5 kDa (Fig. 8A, lane 5). We conclude that the 51- and 48-kDa proteins are the products of the intact *htrA* gene, which is contained between the *PstI*-1 and *SalI* sites on the restriction map (Fig. 3). Removal of the carboxy-terminal coding *PstI*-2–*SalI* DNA fragment from the *htrA* region (Fig. 3) resulted in the production of the 40- and 37.5-kDa HtrA truncated polypeptides.

Two-dimensional electrophoresis of proteins synthesized by *htrA*$^+$ and *htrA* cells was carried out to verify that the 51- and 48-kDa proteins are indeed made from the chromosomal copy under physiological conditions. Results presented in Fig. 9 show that in *htrA*$^+$ cells there is a 48-kDa polypeptide (Fig. 9A and D) that comigrates with the 48-kDa protein that is synthesized in maxicells carrying plasmid pBL133 (Fig. 9E), which is absent in extracts from the *htrA63* mutant (Fig. 9F). This protein is also missing in extracts from mutant *htrA22* cells (data not shown). We conclude that the 48-kDa protein is the *htrA*$^+$ gene product. The 51-kDa precursor

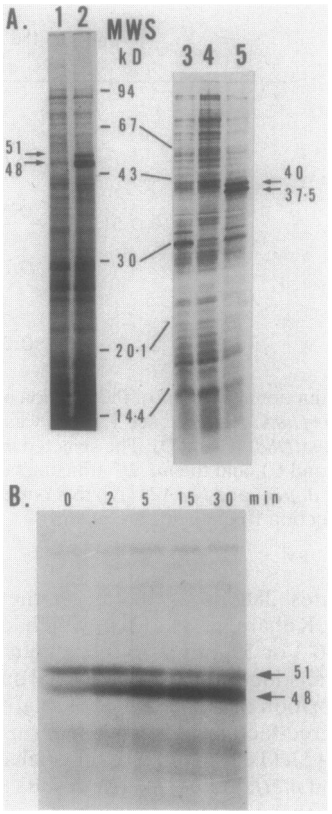

FIG. 8. Expression of the *htrA* gene in maxicells. (A) Proteins synthesized in maxicells carrying pEMBL8$^+$ vector (lanes 1 and 3), pBL133 plasmid (lane 2), pBL13 plasmid (lane 5), and no plasmid (lane 4) were labeled with [^{35}S]methionine for 15 min at 37°C; resolved on 12.5% polyacrylamide gels along with protein molecular weight standards (MWS); stained with Coomassie brilliant blue, and autoradiographed. The arrows point to the positions of the peptides coded by plasmids pBL133 and pBL13. (B) Processing of the HtrA protein in maxicells. Proteins synthesized by maxicells carrying plasmid pBL133 were labeled for 2 min at 37°C with [^{35}S]methionine (lane 1). The incorporation was stopped by the addition of a 200-fold excess of cold methionine for 2, 5, 15, and 30 min (lanes 2, 3, 4, and 5, respectively). Portions were mixed with an equal volume of ice-cold 20% trichloroacetic acid, washed with cold acetone, dried, lysed in sodium dodecyl sulfate sample loading buffer, and electrophoresed as described above for panel A, along with molecular weight standards. An autoradiogram of the gel is shown. The arrows point to the position of the 51-kDa full-length protein and the 48-kDa processed protein.

protein could not be detected in wild-type cells carrying a single copy of the *htrA*$^+$ gene (Fig. 9A and D). There are two possible explanations for the absence of the 51-kDa protein in these cells. Either it is not synthesized or it is rapidly converted to the 48-kDa form. The existence of the 51-kDa protein in cells carrying plasmid pBL133 (Fig. 9G) is in favor of the latter explanation. Since the level of the 51-kDa protein in these cells is much lower than that in maxicells carrying the pBL133 plasmid (Fig. 9E and G), it is probable that the processing in normal cells is very efficient when compared with that in maxicells. This is the reason why the 51-kDa precursor polypeptide could not be found when only a chromosomal copy of the *htrA*$^+$ gene was present.

Expression of the *htrA*$^+$ gene in vitro. The in vitro maxicell experiments were supplemented by in vitro S30 extract coupled transcription-translation experiments. These exper-

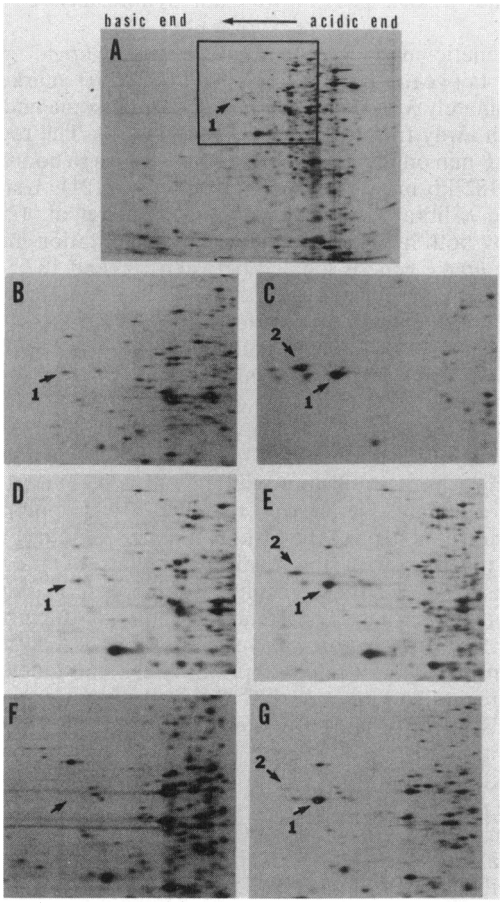

FIG. 9. Synthesis of the *htrA* gene product in *E. coli* cells. Two-dimensional electrophoresis of [^{35}S]methionine-labeled proteins synthesized by B178 *htrA*$^+$ cells (A and D), maxicells carrying the plasmid pBL133 (C) or pEMBL8$^+$ (B) vector, *htrA63* cells (F), and *htrA63* cells carrying plasmid pBL133 (G). Mixed extracts of cells in panels C and D are shown in panel E. Bacteria were labeled for 6 min, which was 5 min following a shift from 30 to 42°C. Maxicells were labeled for 15 min at 37°C. The region shown in a rectangle in panel A corresponds to the areas shown in panels B to G. The arrows point to the position of the precursor 51-kDa HtrA protein (arrow 2) and the processed 48-kDa HtrA protein (arrow 1).

iments showed that a polypeptide of approximately 51 kDa is encoded by the *htrA*$^+$ plasmids pBL133 and pBL34 (Fig. 10) and pBL52 (data not shown). As opposed to the form obtained in the in vitro maxicell experiment, the 48-kDa form was the less abundant one. This could be due to the absence of membranes and, hence, to the lack of processing activity in the S30 extract (see below).

The plasmid pBL13, which carried the smallest insert that was still able to suppress the HtrA$^-$ phenotype, coded for a polypeptide of about 40 kDa (Fig. 10). One explanation for this is that the 40-kDa polypeptide is a truncated form of the larger 51-kDa polypeptide. To confirm this, we analyzed the peptide maps of these polypeptides by the procedure described by Cleveland et al. (8). Briefly, the proteins labeled in an in vitro system with [^{35}S]methionine were separated on a 12.5% polyacrylamide gel; and the gel was fixed, washed with water, dried, and autoradiographed. Relevant radioactive protein bands were excised from the gel, loaded onto a 20% polyacrylamide gel, and overlaid with a buffer containing glycerol and α-chymotrypsin (25 μg/ml) (Fig. 11) or *S. aureus* V8 protease (2.5 μg/ml) (data not shown). Limited digestion of the proteins took place during their slow migration through the concentrating gel. In both cases the peptide

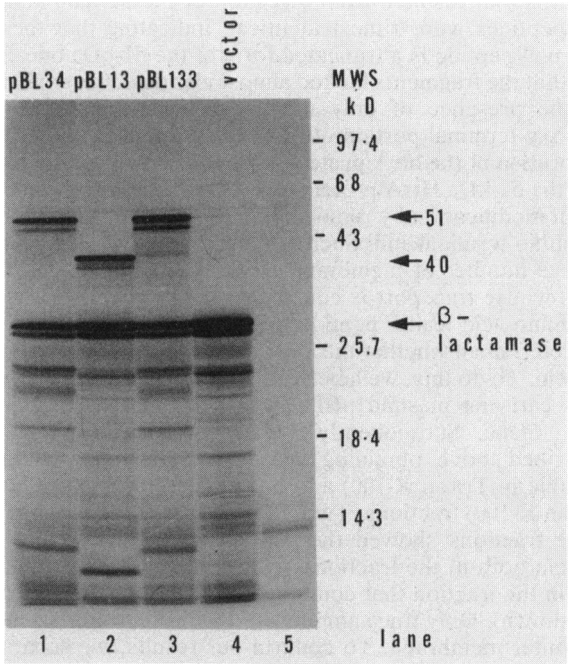

FIG. 10. Expression of the *htrA* gene in vitro. Various plasmids carrying the *htrA* gene region were used to direct polypeptide synthesis in a coupled transcription-translation system. [^{35}S]methionine-labeled proteins were resolved on a 12.5% polyacrylamide gel along with prestained molecular weight standards (MWS). The gel was dried and autoradiographed. The plasmids used were pBL34 (lane 1), pBL13 (lane 2), pBL133 (lane 3), pEMBL8$^+$ vector (lane 4). Lane 5 represents a control without DNA. The arrows indicate the positions of the full-length 51 kDa peptide, the truncated 40-kDa HtrA peptides, and β-lactamase (coded by the vector).

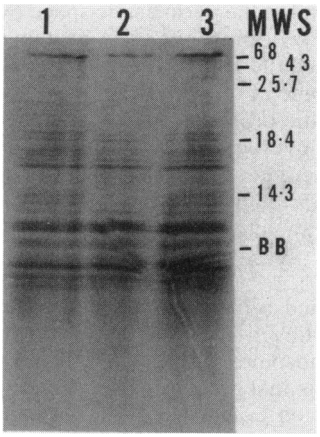

FIG. 11. Peptide mapping of the HtrA polypeptides synthesized in vitro. The full-length 51-kDa polypeptide, coded by plasmid pBL34 (lane 1) or pBL133 (lane 3) and the short 40-kDa polypeptide coded by pBL13 (lane 2) were subjected to limited proteolysis with α-chymotrypsin (25 μg/ml) in the presence of sodium dodecyl sulfate and resolved on 20% polyacrylamide gel along with prestained molecular weight standards (MWS). An autoradiogram of the gel is presented. BB, Position of the bromophenol blue dye.

patterns that resulted from digestion of the 51- and 40-kDa polypeptides were almost identical, indicating that the 40-kDa polypeptide is a truncated form of the 51-kDa one. The fact that the fragments looked almost identical was explained by the presence of only one methionine residue in the carboxy-terminal portion of the HtrA protein (24).

Location of the htrA protein in *E. coli* cells. We concluded that the 51-kDa HtrA protein undergoes rapid posttranscriptional modification by removal of approximately 3 kDa from its amino-terminal end. Such protein processing is typical for a large number of membrane-associated and secreted proteins whose transport is coupled to the removal of a 20- to 40-amino-acid leader peptide (33). Because of this analogy, we determined whether the *htrA* gene product is a membrane protein. To do this, we labeled, with [^{35}S]methionine, *E. coli* cells carrying plasmid pBL133, which contains the entire *htrA*$^+$ gene. Subsequently, we fractionated the cells, as described above, obtaining the cytoplasmic inner membrane (soluble in Triton X-100) and outer membrane (insoluble in Triton X-100) fractions. Two-dimensional electrophoresis of these fractions showed the presence of the 48-kDa HtrA protein both in the fraction which contained the cytoplasm and in the fraction that contained the inner membrane (data not shown). Only trace amounts of the protein were found in the outer membrane. To confirm our results, we separated the total membrane and cytoplasmic fractions by another method by which we used centrifugation in a sucrose gradient, as described by Ito et al. (20). Again, we found the 48-kDa HtrA protein in both the cytoplasmic and membrane preparations (data not shown). We did not perform a control experiment to determine the extent of separation of the cytoplasmic and membrane proteins, so there is a possibility that there was some cross-contamination. However, since the HtrA protein was present in high amounts in both fractions, which were obtained by two different methods, we concluded that the processed 48-kDa HtrA protein, when expressed from a multicopy plasmid, is located both in the cytoplasm and in the membrane. In a separate set of experiments we asked whether the HtrA protein could also be partitioned in the periplasm. To do this we followed the procedure described by Ames et al. (1) for the identification of periplasmic proteins. No significant amount of the HtrA protein was found in the periplasmic space by this procedure (data not shown). Because the HtrA protein is made in small amounts, we used the HtrA-overproducing clone pBL133. It could be that some of the HtrA protein was indeed periplasmic, but when the protein was overproduced, the transport machinery was overwhelmed and the majority of the HtrA protein stayed either in the cytoplasm or the cytoplasmic membrane.

DISCUSSION

We constructed a library of *E. coli* mutants at 30°C by randomly inserting a mini-Tn*10* transposon (40) into the bacterial chromosome. From this library we screened and obtained mutants that grew well at 30°C but that were unable to grow at 42°C on L-agar plates. Two of these mutants, 22 and 63, were shown to have mini-Tn*10* insertions in the same gene, which we called *htrA*. We concluded that these mutations are in the same gene because (i) they possessed very similar phenotypes; (ii) they could not be separated by P1 transduction; (iii) the mini-Tn*10* inserts were found in the same 1.3-kb *Pst*I-*Pst*I chromosomal fragment, although in different sites; (iv) both mutations could be suppressed by the cloned 1.3-kb *Pst*I-*Pst*I chromosomal fragment; and (v) the same 48-kDa protein was not synthesized by either mutant.

By genetic mapping, we found that the *htrA*$^+$ gene is located between the *fhuA* (*tonA*) and *dapD* markers, at approximately 3.7 min on the *E. coli* chromosome and about 0.06 min away from the *optA1* mutation (35). The region at 3.7 to 3.8 min on the genetic map corresponds to coordinates 177 to 182 kb on the *E. coli* physical map (21). Using the genomic λ library constructed by Kohara et al. (21), we found by both hybridization and complementation methods that the *htrA*$^+$ gene is located between 180 and 187.5 kb on the physical map. Therefore, there is very good agreement between our genetic and physical mapping results.

We used the cloned *htrA*$^+$ gene to identify its gene product both in vitro and in vivo. We found that in vitro, the plasmids which carry the *htrA*$^+$ gene code for a basic protein of approximately 51 kDa. However, plasmid pBL13, which carries only a 1.3-kb *Pst*I-*Pst*I fragment of the chromosome, coded for a protein of approximately 40 kDa (Fig. 10). By peptide mapping, we showed that the 40-kDa polypeptide was a truncated form of the 51-kDa full-size protein (Fig. 11). Taken together with the restriction maps of the *htrA*$^+$ plasmids (Fig. 3), these results enabled us to assign the direction of the transcription and translation of the *htrA* gene (Fig. 3). This conclusion was verified by our finding that the *htrA* promoter is located approximately 150 nucleotides downstream from the *Pst*I-1 site and that transcription proceeds toward the *Pst*I-2 site (24). Hence, the truncated 40-kDa polypeptide is missing part of carboxy terminus of the 51-kDa protein (Fig. 3). Furthermore, it appears that this missing protein fragment is not essential for the *htrA* protein function when the protein is overproduced.

In maxicells, the levels of the 48-kDa protein are much higher than those of the 51-kDa precursor because of the rapid processing (Fig. 9C). This difference is more dramatic in normal cells harboring the *htrA*$^+$ plasmid; there were only trace amounts of the 51-kDa protein and high levels of the 48-kDa processed polypeptide (Fig. 9G). This suggests that the processing that occurs in nonirradiated cells is more efficient when compared with that in maxicells and explains why we could only detect the 48-kDa processed protein in normal cells that carried only a single chromosomal copy of the *htrA*$^+$ gene (Fig. 9D). The fact that the 48-kDa protein was missing from extracts of the *htrA* cells (Fig. 9F) supports our conclusion that this is indeed the *htrA* gene product.

It is known that many membrane proteins are synthesized in a precursor form that contains an N-terminal leader peptide consisting of 20 to 40 amino acids which is cleaved on insertion of the protein into the membrane (33). Our finding that the 51-kDa *htrA* protein is processed by removal of about 3 kDa from its amino-terminal end and that the processed 48-kDa protein is found in the cytoplasmic membrane is consistent with the hypothesis that the transport of the *htrA* protein to the cytoplasmic membrane is accompanied by cleavage of the leader peptide. This conclusion is also supported by the fact that very little HtrA protein processing was observed in the in vitro system, which contained no membranes and probably only a small amount of leader peptidase. Other membrane-transported proteins are also synthesized mainly in a precursor form in vitro (28).

We do not have any biological clues as to the exact role that the HtrA protein plays in *E. coli* growth and physiology. Our finding that the addition of 2.5 mM serine can alleviate the Tr$^-$ phenotype caused by the *htrA* mutation when cells are grown on minimal medium suggests that the *htrA* gene product may somehow be involved in cellular osmoregula-

tion, since it is known that serine is an osmoprotecting agent (23). To test this, we investigated the effects of other osmoprotectants (betaine, choline, and dimethylglycine) and found that they did not have any influence on the HtrA$^-$ phenotype (unpublished data). The addition of leucine to the medium also protected the *htrA* mutant cells at high temperatures on minimal plates. Since these two amino acids are very different in terms of structure, biosynthesis, and transport, an explanation of their similar functions requires further investigation.

A possibility that cannot be completely eliminated from our data is that the *htrA* insertion mutants died at elevated temperatures because of a toxic effect exhibited by the HtrA22 and HtrA63 truncated proteins. This toxic effect could be caused by the jamming of some component of the protein secretion machinery, which is more pronounced at 43°C because of the higher levels of *htrA* gene expression at this temperature (24). Arguments against this possibility include the facts that (i) cloning of the *htrA22* and *htrA63* mutations on high-copy-number plasmids does not lead to the death of *E. coli* (24) and (ii) both the 40-amino-acid HtrA63 polypeptide and the 308-amino-acid HtrA22 polypeptide result in *E. coli* inviability at 43°C, whereas a 376-amino-acid mutant HtrA polypeptide (produced by a truncated *htrA* gene) retains biological activity (24).

ACKNOWLEDGMENTS

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We thank Jeni Urry for cheerful and expert editing of the manuscript.

ADDENDUM IN PROOF

Following a suggestion by Graham Walker and Bernd Bukau, we have exchanged information and strains with K. Strauch and J. Beckwith and have concluded that the *htrA* and *degP* (Proc. Natl. Acad. Sci. USA **85**:1576–1580, 1988) genes are identical (K. Strauch, K. Johnson, and J. Beckwith, submitted for publication).

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