Identification of the *Escherichia coli* sohB Gene, a Multicopy Suppressor of the HtrA (DegP) Null Phenotype

LISA BAIRD,* BARBARA LIPIŃSKA,† SATISH RAINA, AND COSTA GEORGPOULOS

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

Received 20 May 1991/Accepted 1 July 1991

We cloned and sequenced the sohB gene of *Escherichia coli*. The temperature-sensitive phenotype of bacteria that carry a Tn10 insertion in the htrA (degP) gene is relieved when the sohB gene is present in the cell on a multicopy plasmid (30 to 50 copies per cell). The htrA gene encodes a periplasmic protease required for bacterial viability only at high temperature, i.e., above 39°C. The sohB gene maps to 28 min on the *E. coli* chromosome, precisely between the topA and btuR genes. The gene encodes a 39,000-Mr precursor protein which is processed to a 37,000-Mr mature form. Sequencing of a DNA fragment containing the gene revealed an open reading frame which could encode a protein of Mr 39,474 with a predicted signal sequence cleavage site between amino acids 22 and 23. Cleavage at this site would reduce the size of the processed protein to 37,474 Mr. The predicted protein encoded by the open reading frame has homology with the inner membrane enzyme protease IV of *E. coli*, which digests cleaved signal peptides. Therefore, it is possible that the sohB gene encodes a previously undiscovered periplasmic protease in *E. coli* that, when overexpressed, can partially compensate for the missing HtrA protein function.

*Escherichia coli* undergoes a heat shock response upon a shift to high temperature. The heat shock response involves the induction of a number of proteins, known as heat shock proteins (for a review, see reference 26). Classical heat shock genes are members of the σ2 regulon because their transcription is positively regulated by the σ2 subunit of RNA polymerase (9, 25, 26). The σ2 subunit is the product of the rpoH (htpR) gene (9).

Recently, a new heat shock regulon has been discovered. The transcription of members of this regulon is positively regulated by a new σ factor, known as σ24 (σ4) (4, 39). So far, the only two genes which are known to be transcribed by σ24 are rpoH and htrA (degP) (4, 5, 20, 39). The htrA gene was discovered independently by two different approaches. One approach involved a search for genes whose products are required at high temperature (above 42°C) but are not required at low temperature (30°C) (19). The other strategy involved the characterization of a mutant which was defective in proteolysis of certain periplasmic fusion proteins (33). Subsequent studies verified that the product of the htrA gene is indeed an endopeptidase (21). Therefore, the product of the htrA gene is a periplasmic protease that is required only at elevated temperatures.

In an effort to understand more fully the function of the htrA gene product, we have begun an extensive analysis of extragenic suppressors of the temperature-sensitive phenotype of htrA mutant bacteria. Previously, we described the characterization of two cold-sensitive suppressor mutations, mapping in a gene which we called sohA (suppressor of htrA) (2). The sohA gene has since been shown to be identical to the prfA gene, whose product is involved in protein export from the cytoplasm (14). Here we report the characterization of a gene, sohB, which can suppress the temperature-sensitive phenotype of htrA mutant bacteria when present in the cell on a multicopy plasmid.

**MATERIALS AND METHODS**

**Media.** All strains were grown in L broth or on L agar plates (1). When necessary, media were supplemented with ampicillin at 50 μg/ml, kanamycin at 50 μg/ml, tetracycline at 15 μg/ml, or chloramphenicol at 20 μg/ml. The low-sulfur minimal medium, used when labeling proteins, has been described previously (32).

**Bacterial strains, plasmids, and bacteriophages.** Bacterial strains and plasmids are described in Table 1. The mini-Mu replicon element Mud5294 was used for the in vivo cloning of sohB (8). The Kohara library of overlapping λ transducing phages has been previously described (16). The kanamycin resistance (Kan') cassette, used to make an insertion in sohB, was isolated from plasmid pUC4-KSAC (Pharmacia) on a 1.3-kbp PstI DNA fragment which contains the Tn903 Kan' gene. Pl transductions were done using standard techniques (24).

**Cloning of sohB.** The multicopy suppressor gene sohB was cloned by using the in vivo mini-Mu plasmid cloning technique, as described previously (7). However, we grew our mini-Mu lysate on a Mud5294-carrying strain which also carried a Tn10 insertion in the chromosomal htrA gene. This was done to prevent simply recloning the htrA gene. htrA63 mutant bacteria, strain BL20, were then infected with this lysate and plated on L-kanamycin plates at 42°C. Mini-Mu plasmids were isolated from colonies which formed at 42°C, and one plasmid was chosen for further study (pLB630).

**Subcloning and construction of deletions.** All DNA manipulations were done by standard procedures (23). T4 DNA ligase and restriction enzymes were purchased from Boehringer Mannheim Biochemicals and were used according to the manufacturer's specifications. Deletions were made with DNase I as described previously (12) or with exonuclease III as described previously (11).

**Mapping.** Mapping was done by hybridization to the
Kohara library of \( \lambda \) transducing phages with overlapping bacterial DNA inserts as described previously (19).

**DNA sequencing.** All DNA sequencing was done with the Sequenase system from United States Biochemical Corp. The entire sequence was determined for both strands of DNA. Computer analysis of DNA and protein sequences was done with the Intelliconics, Inc., PC/Gene program package. The SohB protein sequence was compared with the SWISS-PROT data base (release no. 15) using the FASTA method of Pearson and Lipman (29).

**Labeling of plasmid-encoded proteins.** Directed DNA restriction fragments were first cloned into the pBluescript KS(+) and SK(+) vectors from Stratagene. These two vectors are identical except for the orientation of a T7 polymerase promoter with respect to their polylinkers. Plasmid-encoded proteins were then labeled with \( ^{35} \)S protein-labeling mix Expre\(^{35S} \) (Dupont, NEN Research Products) with a T7 polymerase expression system as described previously (35). For the pulse-chase experiment, the cells were pulsed for 1 min with label, and an aliquot was removed at 1 min to 0.1 volume of ice-cold 50% (w/vol) trichloroacetic acid. The rest of the culture was then chased with a 100-fold excess of cold methionine, and aliquots were subsequently removed at 1, 4, and 9 min, after the start of the chase, to 0.1 volume of ice-cold trichloroacetic acid. Cell extracts were electrophoresed on 12.5% sodium dodecyl sulfate-polyacrylamide gels, essentially by the method of Laemmli (18).

**Inactivation of the chromosomal sohB gene.** The method of Oden et al. (27) was used to move the Kan\(^{'\text{a}}\) cassette, inserted in the PstI site of the sohB gene in pLB789, to the sohB gene in the chromosome. This method is based on the observation that in a recB recC sbcA sbcB background, selection for only the antibiotic resistance carried by an insertion element in the genomic region of an introduced plasmid reveals events in which the insertion element has recombined onto the chromosome. We transformed strain JC7623 with plasmid pLB789 and selected for Kan\(^{'\text{a}}\), the antibiotic resistance encoded by our cassette. Five Kan\(^{'\text{a}}\) colonies grew, and four of these five were now ampicillin sensitive (Ap\(^{\text{a}}\)), presumably owing to loss of the plasmid. Southern analysis by standard techniques (23) was done on one of these Kan\(^{'\text{a}}\) Ap\(^{\text{a}}\) isolates to verify that the Kan\(^{'\text{a}}\) cassette had indeed been recombined into the sohB gene on the chromosome. Hybridization of the nylon membrane (Amersham) was used according to the manufacturer’s specifications, and DNA probes were radioactively labeled with \( ^{32} \)PdATP (NEN Research Products) by using the Prime It Random Primer Kit (Stratagene), again according to the manufacturer’s specifications.

**Nucleotide sequence accession number.** This sequence has been given the GenBank accession number M73320.

### RESULTS

**Cloning, subcloning, and mapping of sohB.** Mini-Mu plasmid pLB630 was isolated on the basis of its ability to reverse the temperature-sensitive phenotype of htraA mutant bacteria at 42°C. This Mini-Mu plasmid was isolated from a mini-Mu lysate which was grown on a bacterial strain which carried a Tn10 insertion in the htra gene. The rationale for using this lysate was to prevent simply recloning the htra wild-type gene but instead to possibly clone another gene, which when present on a multicopy plasmid could rescue the temperature sensitivity of the htra mutant bacteria.

Plasmid pLB630 DNA and plasmid DNA from a random mini-Mu selected at 30°C and used as a control were isolated and retransformed into strain BL20 to verify that pLB630 could indeed rescue the temperature-sensitive phenotype. Bacteria transformed with pLB630 DNA were observed to survive better at 42°C on L-kanamycin plates than bacteria transformed with a control random mini-Mu plasmid, although their growth rate was very slow (see below).

To subclone the suppressor gene, pLB630 DNA was partially digested with the restriction endonuclease Sau3A and the resulting DNA fragments were ligated into the vector pBR325. The vector pBR325 has the phage number (30 to 50 copies per cell) as the mini-Mu plasmid. A subclone, pLB669, was obtained which could still rescue the temperature-sensitive phenotype of strain BL20. Restriction mapping of pLB669 revealed that it carried a 7-kbp DNA insert (Fig. 1A).

To map the bacterial DNA insert in pLB669 on the E. coli chromosome, we hybridized nick-translated pLB669 DNA to a nylon filter containing plaque-lifted DNA from each of the \( \lambda \) transducing phage in the Kohara library. The 476 \( \lambda \) transducing phage in this library carry bacterial DNA inserts that span the entire E. coli chromosome. Positive hybridization was obtained with phage 242 (4D1), 243 (2A3), 247 (4D10), 253 (4F1), and 254 (13F9) (data not shown). The inserts in phage 242, 243, and 247 are located at approximately 25 min on the E. coli chromosome. The inserts in phase 253 and 254 are located at approximately 28 min. The restriction map of pLB669 matches the restriction map of E. coli DNA carried by phage 253 and 254 as reported by Kohara (data not shown). We do not know why positive hybridization is also observed with the other three phage in our Kohara phage collection, although contamination of these lysates or chromosomal rearrangements in either our strain or Kohara’s strain are possible explanations. Another possible explanation is that these Kohara phage, which carry bacterial DNA inserts from the 25-min region, carry an E. coli gene(s) which shares homology with sohB. Indeed, the sohB gene does share homology with the sspa gene of E. coli (see below), but the sspa gene is located at 34.5 min and not at 25 min where the bacterial DNA was carried by the cross-hybridizing Kohara phage is located.

The genes topA (37) and btuR (22) both map to the 28-min region of the E. coli chromosome, and through restriction enzyme analysis, we were able to place both of these genes on our subclone pLB669 (Fig. 1A). To determine whether
the topA gene was responsible for suppression of the temperature-sensitive phenotype, we constructed subclone pLB709. Plasmid pLB709 consists of a 4-kbp SphI DNA insert in the SphI site of pBR322. This subclone still retains the ability to rescue htrA mutant bacteria at 42°C, although it no longer carries the topA gene (Fig. 1A). To determine whether the btuR gene was responsible for the complementation ability, we constructed DNase I deletions of pLB709 and obtained plasmids pLB740 and pLB744. Plasmid pLB740 still retains the btuR gene, but it has lost complementation ability. On the other hand, plasmid pLB744 retains approximately 2 kbp of DNA immediately upstream of btuR and retains complementation ability. In addition, we also constructed plasmid pLB789 by inserting a Kan' cassette into the PstI site located approximately 1.5 kbp upstream of the btuR gene, and this also abolished complementation ability. Thus, the suppressor gene was very precisely mapped between the btuR and topA genes at 28 min on the E. coli chromosome.

Inactivation of the chromosomal copy of the sohB gene. The Kan' cassette from pLB789 was crossed onto the E. coli chromosome in strain JC7623 by the method of Oden et al.

FIG. 1. Restriction map and proteins encoded by the E. coli DNA fragment containing the sohB gene. (A) The name of each subclone is indicated to the right of each line which represents the insert that it carries. The + or - symbol at the far right indicates the ability of each plasmid to complement the temperature-sensitive phenotype of strain BL20. The dotted lines indicate that the particular DNA sequences are derived from pBR325 or pBR322. The arrows represent the positions of the topA, btuR, and an unknown gene (designated with a ?). (B) A T7 polymerase expression system was used to selectively label plasmid-encoded proteins as described in Materials and Methods. Cell extracts were then electrophoresed on a sodium dodecyl sulfate-12.5% polyacrylamide gel. The plasmid carried by each cell in each extract is indicated at the top of each lane. Plasmids pLB771 and pLB772 carry the 4-kbp BamHI-SalI-directed DNA fragment from pLB709 now cloned into the BamHI-SalI sites of pBluescript KS and SK, respectively. Plasmid pLB773 and pLB774 carry the slightly smaller 3-kbp PstI-SalI-directed DNA fragment from pLB709 now cloned into the PstI-SalI sites of pBluescript KS and SK, respectively. The numbers to the right indicate the positions and sizes in daltons of protein molecular size standards.
Kan' Ap^6 colonies were isolated, which presumably have recombined the Kan' marker into the sohB gene on the chromosome with subsequent loss of the Ap^6 plasmid. We then constructed strain LB849 by transducing out the Kan' cassette with phage P1 back into the parental strain B178. To verify that the Kan' cassette was indeed located within the chromosomal copy of the sohB gene, chromosomal DNA from the parental strain, B178, and the Kan' strain, LB849, was digested with the restriction enzyme EcoRV, transferred to nylon membrane, and probed with a radioactively labeled 1.3-kbp EcoRV DNA fragment containing most of the sohB gene. This DNA probe hybridized to a 1.3-kbp EcoRV DNA band in the B178 chromosomal DNA and a 2.6-kbp EcoRV DNA band in the chromosomal DNA isolated from strain LB849 (data not shown). Since the Kan' cassette is 1.3 kbp in size, this new DNA band of 2.6 kbp is precisely the correct size if the insertion was indeed located within the chromosomal 1.3-kbp EcoRV fragment. Thus, the Kan' cassette in strain LB849 is indeed located within the sohB gene.

We then tested whether strain LB849 had acquired a temperature-sensitive phenotype resulting from the insertional inactivation of the sohB gene. We observed no change in the ability of LB849 bacteria to grow on L plates at 30, 37, 42, or 43°C relative to wild-type B178 bacteria. Thus, the sohB gene is not essential for bacterial viability, and bacteria which carry an insertion in the sohB gene do not become temperature sensitive.

Viability curves. As stated above, the ability of our mini-Mu plasmid to complement the temperature-sensitive phenotype of htrA mutant bacteria on L plates was weak but nevertheless reproducible. Throughout the subcloning steps described above, the level of complementation remained the same. On L agar plates at 42°C, small colonies were visible after approximately 36 to 48 h when strain BL20 was transformed with pLB699, pLB709, or pLB740. These small colonies were equal in number to those colonies which grew on a control plate incubated at the permissive temperature of 30°C. When transformed with pBR322 alone or pLB744, virtually no colonies survived an incubation at 42°C. An interesting phenotype was observed when the growth kinetics of the small bacteria was monitored in liquid culture. When cultures of strain BL20 carrying any of the above plasmids were shifted from 30 to 42°C, the optical density declined and the bacteria began to lyse (data not shown). However, if the number of viable cells in each of the cultures was monitored, an interesting result was obtained (Fig. 2). If the cells carried either pLB709 or pLB744, they died at a slower rate at 42°C than the cells carrying pBR322 or pLB789. After 2 to 3 h of incubation at 42°C, there were approximately ten times as many viable cells in the culture if the htrA mutant bacteria were carrying pLB709 or pLB744 (Fig. 2B). Thus, rather than fully compensating for the loss of htrA function, the multicopy suppressor gene perhaps allows more cells to survive for a longer time at the nonpermissive temperature of 42°C, in liquid media. Suppression may be better on plates than in liquid because the temperature of the plates slowly rises to 42°C, rather than being raised immediately to 42°C as in liquid, and this may give the cells more time to adapt to the higher temperature.

Identification of sohB gene product. To identify the proteins encoded by the 4-kbp SphI DNA insert in plasmid pLB709, we used the restriction enzyme sites BamHI and SalI to move the DNA insert to the plasmids pBluescript KS and SK. The BamHI and SalI sites are located in pBR322 DNA sequences on either side of the SphI site, which enabled us to move the insert to the BamHI-Sall sites of KS and SK as a directed DNA fragment (Fig. 1A). The 4-kbp BamHI-Sall DNA fragment in the vector KS is plasmid pLB771, and the same fragment in the vector SK is plasmid pLB772. The vectors pBluescript KS and SK are identical except for the orientation of a T7 polymerase promoter with respect to their polyclinker, thus allowing the identification of any proteins encoded by an insert in a specific orientation. The T7 polymerase labeling system revealed the presence of two proteins of approximate M, 22,000 and 25,000 encoded by pLB771 (Fig. 1B, lane 3). When the insert was transcribed in the opposite orientation in plasmid pLB772, a single protein of M, 37,000 was observed (Fig. 1B, lane 4). We then constructed another set of plasmids, pLB773 and pLB774, which carried the smaller 3-kbp PstI-Sall DNA fragment from pLB709 in the vectors KS and SK, respectively. Plasmid pLB773 still encoded the 22,000- and 25,000-M, proteins, but the protein produced by pLB774 was
The sequence of the topA gene, reported by Tse-Dinh and Wang (37), includes 1.2 kbp of upstream sequence. These investigators noted the presence of an ORF which they speculated might be the carboxyl terminus of an unknown gene immediately adjacent to topA. Our sequencing revealed that the ORF which they described is the sohB gene, although we found some differences with their published sequence, one of which results in a frameshift near the carboxyl end of their putative protein that lengthens the actual ORF by approximately 300 bp. Therefore, the actual distance between the end of the sohB gene and the beginning of the topA gene is not 900 bp, as they speculated, but is actually only 600 bp.

Computer analysis of the amino acid sequence of the sohB gene product revealed some interesting predictions about the protein. As mentioned above, there is a potential signal sequence cleavage site between amino acids 22 and 23, predicted by the program PSIGNAL (38). In addition, a potential transmembrane spanning domain was predicted from amino acids 190 to 210 (underlined in Fig. 4) by three different computer programs that predict the position of membrane-associated helices. The computer programs used for this prediction were HELIXMEM (3), RAOARGOS (30), and SOAP (15). A hydrophobicity plot for the SohB protein is shown in Fig. 5. An extremely hydrophobic region is found at the amino terminus of the protein, which is the predicted signal sequence, and another hydrophobic region is shown in the central portion of the protein around amino acids 190 to 210 as noted above.

A comparison of the SohB protein sequence with the SWISS-PROT library revealed the presence of regions of strong homology with the product of the sppA gene of E. coli and the product of the vcaC morphogenetic gene of bacteriophage λ (Fig. 6). The sppA gene of E. coli encodes the inner membrane enzyme protease IV, which digests cleaved signal peptides (13). The vcaC gene of bacteriophage λ encodes the minor capsid protein precursor C (6). The SohB protein had 26% identity over a 179-amino-acid stretch with the SppA protein. A relaxation to include conservative amino acid changes increased the similarity to 39%. Likewise, the SohB protein had 25% identity over the same 179-amino-acid stretch with the λ C protein. Again, a relaxation to include conservative amino acid changes increased the similarity to 37%.

**DISCUSSION**

Bacteria which carry a Tn10 insertion in the htrA gene are temperature sensitive, i.e., they are unable to grow at temperatures above 39°C. Other workers discovered the htrA gene independently using a different approach, and they named the gene degP (33). Their work involved the characterization of mutants defective in the proteolysis of certain periplasmic fusion proteins (34). Thus, the product of the htrA (degP) gene is a periplasmic protease that is required only at elevated temperature.

In this work, we report the cloning and sequencing of a previously undiscovered gene in E. coli that we call sohB (suppressor of htrA). When the sohB gene is present on a multicopy plasmid (30 to 50 copies per cell), htrA mutant bacteria carrying this plasmid can survive at 42°C. Therefore, the sohB gene is a multicopy suppressor of the temperature-sensitive phenotype of htrA mutant bacteria. The suppression is weak and appears to function better on L plates than in L broth. We do not know why this is the case, although one possibility is that a shift to higher temperature

---

**FIG. 3.** Pulse-chase labeling of the SohB protein. Pulse-chase labeling of bacterial cultures was done as described in Materials and Methods. The first two lanes are cell extracts after a 1-min pulse with 35S-protein-labeling mix Expre35S35S, and the subsequent sets of lanes are extracts after 1, 4, and 9 min of chase with excess cold methionine. The plasmid carried by the cells in each extract is indicated at the top of each lane. The numbers to the right indicate the positions and sizes in daltons of protein molecular size standards.

Reduced in size from 37,000 to 20,000  M, (Fig. 1B, lanes 5 and 6). A Kan cassette inserted into this same Pstl site abolished the ability of plasmid pLB797 to complement. Thus, the protein which is responsible for complementation spans the Pstl site and has an  M, of 37,000.

To see whether this 37,000-M, protein might be a processed product of a larger precursor protein, we did pulse-chase experiments with cultures carrying plasmid pLB772. After a 1-min pulse, a protein of approximate  M, 39,000 was seen which was then chased into a protein of approximate  M, 37,000 (Fig. 3, lanes 1, 3, 5, and 7). Thus, the product of the sohB gene is processed, and therefore it is likely that it is exported from the cytoplasm.

**DNA sequencing.** The DNA sequence was determined for the region upstream of the topA gene (Fig. 4). An open reading frame (ORF) was found with a GTG initiation codon at nucleotide 408 which could encode a protein of  M, 39,474. This ORF was transcribed in the same orientation as the 37,000-M, protein seen in the T7 polymerase labeling system with bacteria carrying pLB772 (Fig. 1, lane 4). Computer analysis of the protein sequence with the PSIGNAL program of Von Heijne (38) revealed a putative signal sequence cleavage site between amino acids 22 and 23 (Fig. 4). This would reduce the predicted  M, of the protein to 37,474, which is in precise agreement with the protein observed in the pulse-chase experiment with bacteria carrying pLB772 (Fig. 3, lanes 1, 3, 5, and 7).
FIG. 4. Nucleotide sequence of the sohB gene. The amino acid sequence of the SohB protein is shown below the DNA sequence, starting with the potential GTG initiation codon at nucleotide position 408. The asterisk between amino acids 22 and 23 indicates the potential site of cleavage of a leader sequence. The underlined amino acids, residues 190 to 210, indicate the position of a potential transmembrane-spanning region (see Results). The two arrows, downstream from the stop codon, indicate the position of an inverted repeat sequence that may function as a rho-independent termination signal.

in L broth results in a sudden change to the higher temperature, whereas a shift to a higher temperature on an L plate results in gradual change to the higher temperature. In addition, the embedding of htrA mutant bacteria in agar may allow for better protection against cell lysis at the nonpermissive temperature.

The sohB gene maps at 28 min on the E. coli chromosome precisely between the topA (37) and betaR (22) genes. The sohB gene does not appear to be essential, and bacteria which carry a Kan’ cassette in the gene do not become temperature sensitive. The sohB gene product has an Mr of 39,474 and is processed to a protein with an approximate Mr of 37,000. The product of the htrA gene is also processed (20). In addition, the SohB protein contains one predicted transmembrane-spanning domain; therefore, it is possible that it is an integral membrane protein.

The SohB protein has 39% similarity over one-half of its length with the product of the sppA gene of E. coli. The sppA gene codes for protease IV, a signal peptide peptidase found in the cytoplasmic membrane that digests cleaved signal peptides (13, 36). The sppA gene is also not essential for bacterial growth (36). Bacteria which carry a deletion of the gene digest signal peptides at a considerably slower rate than sppA+ bacteria, although this digestion is still significant (36). This observation suggests that there is another protease(s) involved in signal peptide digestion in the cell envelope. The fact that the SohB protein has homology to protease IV is very interesting since the sohB gene was isolated as a multicopy suppressor of mutations in the htrA gene, which encodes another known periplasmic protease. In addition, Pacaud (28) reported the presence of two membrane-bound proteases in E. coli, one of which had an
apparent $M_r$ of 34,000, which is approximately the same as the $M_r$ of the sohB gene product. We do not know whether we have cloned the 34,000-$M_r$ protease which characterized, but the similarity in size between our protein and hers is suggestive.

Strauch and Beckwith (33) have observed that the temperature-sensitive phenotype of htra ($degP$) mutant bacteria is relieved when the bacteria also carry a mutation causing lipoprotein deficiency. The mutation causing lipoprotein deficiency results in the release of periplasmic proteins into the medium, and the temperature-sensitive phenotype of htra mutant bacteria is relieved. Therefore, the temperature-sensitive phenotype may be due to the accumulation of a toxic protein(s). If the htra protein is indeed a protease, it is possible that overproduction of the protein can somehow compensate for the loss of the Htra protease. Of course, we have not demonstrated that the htra protein indeed possesses the ability to function as a protease, but it is tempting to speculate that the Htra and SohB proteins have overlapping proteolytic functions.

The SohB protein also has 37% similarity over one-half of the length of the protein to the $\alpha$ C protein (6). The region of the SohB protein which is similar to that of $\alpha$ C is the same region of the protein which is similar to the sppA gene product. The $\lambda$ C protein is needed for proper $\lambda$ head morphogenesis (6). The exact role of $\lambda$ C in head morphogenesis is not known, although aberrant prophages are made after $\lambda C^-$ bacteriophage infection of $sup^-$ bacteria (6). It is known that the maturation of prophages involves the participation of each $\lambda$ C molecule in a fusion-cleavage reaction with a $\lambda$ E protein molecule (10). Also, cleavage of $\lambda$ B and degradation of the $\lambda$ Nu3 scaffold protein do not occur if $\lambda$ C is missing (6, 31). It is possible that $\lambda$ C has protease activity and that the homology it shares with the SohB protein is due to the fact that it also is a protease participating in the processing of the $\lambda$ E, $\lambda$ B, and/or $\lambda$ Nu3 proteins.

The mechanism of suppression of the temperature-sensitive phenotype of htra mutant bacteria by overproduction of the SohB protein remains unknown, although evidence suggests that it is a transmembrane protein and might be a periplasmic protease. Further studies will be needed to demonstrate whether the SohB protein does indeed function as a protease in the cell envelope of E. coli.

**ACKNOWLEDGMENT**

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

**REFERENCES**


