The Escherichia coli supX locus is topA, the structural gene for DNA topoisomerase I
(nonsense mutations/cluster mutant alleles/restriction mapping/antigenic determinants/peptide fragments)

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ABSTRACT Mutations in the supX locus, which result in the absence of DNA topoisomerase I enzyme activity in both Salmonella typhimurium and Escherichia coli, are all selected as suppressors of the leu-500 promoter mutation in S. typhimurium. To determine whether the supX locus is the structural gene topA for the DNA topoisomerase I enzyme or is a positive-acting regulator/activator gene for a nearby topA structural gene, nonsense mutations were selected in the E. coli supX gene on an F' episome in S. typhimurium cells.

The cysB-topA region of the episomes with nonsense-mutant supX alleles were then cloned onto plasmid pBR322 and transformed into E. coli cells lacking a chromosomal supX gene. Three such E. coli strains, each carrying cloned DNA from episomes with different nonsense-mutant supX alleles, all lacked DNA topoisomerase I activity but expressed antigenic determinants specific to the enzyme; control cells lacked both enzyme activity and antigenic determinants. Maxicell studies of plasmid-coded proteins demonstrated the absence of the DNA topoisomerase I protein (100 kDa) in the three strains but the appearance of a new smaller peptide in each (36, 47, and 64 kDa). These new peptides must represent fragments of the enzyme resulting from translation termination at the supX nonsense codons and confirm the interpretation that the supX gene is topA, the structural gene for DNA topoisomerase I.

The Salmonella typhimurium supX locus (originally termed su leu 500) is defined as the site of both point and deletion mutations that suppress the leucine auxotrophy imposed by the leu-500 promoter mutation (1, 2). In addition to suppressing promoter mutations, including Escherichia coli lac promoter mutations, all mutations that inactivate the supX gene are highly pleiotropic, affecting the expression of many genes (2–6). The supX locus of both S. typhimurium and E. coli lies between the cysB and trp genes (1, 7) and, in S. typhimurium, close to cysB (2, 8). Restriction mapping of E. coli DNA placed the topA gene close to cysB (9).

Sternlanz et al. (10) described evidence that a gene determining the presence of DNA topoisomerase I in E. coli was linked to the trp operon. Subsequently, Sternlanz et al. (11) and, independently, Truksis and Depew (12) and Truksis et al. (13) concluded that a gene specifying the synthesis of the DNA topoisomerase I enzyme maps at about the same chromosomal location as supX and that mutations of supX result in the absence of that enzyme. Both groups suggested that supX is the structural gene for DNA topoisomerase I and that the gene be renamed top or topA. Truksis et al. (13) also noted that although most supX deletion mutant strains lacked antigens specific to the enzyme, two such deletion strains did produce reduced levels of DNA topoisomerase I antigen. Wang and Becker (9) reported that in E. coli lacking DNA topoisomerase I activity, the presence of a plasmid-borne DNA fragment from the chromosome region between cysB and trp, although too small (2.3 kilobases) to code for the 100-kDa enzyme (9), engendered a positive response to serological tests for DNA topoisomerase I antigenic determinants. These findings strongly support the conclusion that the structural gene for DNA topoisomerase I resides in the chromosome region between the cysB and trp loci. The DNA topoisomerase I enzyme plays an important role in determining the degree of DNA supercoiling (14, 15). A variety of in vivo and in vitro studies, reviewed by Smith (16) and Drlica (15), have demonstrated that the degree of DNA supercoiling affects the expression of many genes, and the correlation with the observation that supX mutations affect the expression of many genes (2–6) is striking.

All of the above evidence is consistent with the interpretation that supX is the structural gene for topoisomerase I but it does not preclude the possibility that the supX locus is some type of activator/regulator gene (17) coding for a product involved in initiating expression of a nearby topA structural gene. The studies described here were designed to resolve these alternatives. The experimental strategy involved construction of an S. typhimurium–E. coli hybrid with a leu-500 promoter mutation and a supX deletion mutation on the S. typhimurium chromosome and an E. coli supX allele on an F123 episome. Clones with mutations of the E. coli supX gene would then be selected by their suppression of the leu-500-caused leucine auxotrophy, followed by identification of several which were nonsense mutations of independent origin and resulted in a lack of DNA topoisomerase I activity. Since the nonsense mutations would act as termination points for translation, the products of the mutant supX alleles should consist of peptide fragments terminating at the sites of the nonsense codons. The absence of the DNA topoisomerase I protein and the appearance of peptides representing fragments of the enzyme would demonstrate that the supX nonsense mutations were located in the structural gene for the enzyme. The evidence presented here in our analysis of nonsense mutant alleles of the E. coli supX gene and their peptide products validates the conclusion that supX is the structural gene for DNA topoisomerase I.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The S. typhimurium strains are all derived from strain LT2. The introduction of the E. coli F123 episome (18) into the Salmonella strains was accomplished as described (7). Plasmid pBR322 (19) served as the cloning vehicle for the cysB topA segments of supX mutant F123 episomes from S. typhimurium strains.

Abbreviation: bp, base pair(s).
The _E. coli_ strains used were DM800 and DM700 (11), each bearing a deletion of _supX_ extending into _cysB_, Δ(_supX-cysB_), of independent origin. Both the DM700 and DM800 deletions, originally on F'123 _E. coli_ episomes, were selected in _S. typhimurium_ as suppressors of _leu-500_, transferred back to _E. coli_, and introduced into the _E. coli_ chromosome by transduction (D. Mascarenhas, personal communication). A third _E. coli_ strain was streptomycin-resistant (Str SM) KY1303: thi-1 argE3 proA2 galK2 lacY1 mtl-1 xyl-5 tsa-29 supE44 rpsL λ Δ(_supX-cysB_); the latter deletion was derived from DM700. All three _E. coli_ strains lacked DNA topoisomerase I activity due to the _supX_ deletions.

**Media.** _S. typhimurium_ strains were grown in minimal synthetic medium with 0.2% glucose and appropriate supplementation to supply nutritional requirements or carry out selections for specific phenotypes as described (7). To select the _opp_ mutations tri-L-ornithine, obtained from Miles-Yeda (Rehovot, Israel) was used to inhibit growth of _opp_+ cells on minimal synthetic medium (20). Sodium citrate was omitted from the medium when testing for sensitivity to chromium ion, to detect _tonB_ mutations (21). Complete liquid medium for routine growth of cultures was Luria broth (22).

**Transduction and Conjugation.** Transductions were carried out as described (23) but with lysates of _E. coli_ mutant phage HT105/1 grown on the donor strains. Conjugations were carried out by plating cells of the donor and recipient cultures together on agar media that lacked a nutritional supplement required by the donor but was appropriately supplemented to allow growth of a recipient cell that received the genes carried on the F'123 episome of the donor.

**Mutagenesis to Induce _supX_ Single Base-Pair Transition Mutations.** Minimal medium supplemented with leucine, histidine, and tryptophan and containing 300 μg of 2-amino-propionitrile per ml was used for a series of cultures, each inoculated from a single-colony isolate of strain PM801 and grown 36 hr at 37°C with aeration. Samples of each fully grown culture were spread on minimal synthetic agar supplemented with tryptophan and histidine but not with leucine. Those _Leu_+ colonies arising because of mutations in the _supX_ gene were initially identified by their smaller size (1) after 48 hr of incubation at 37°C.

**Cloning the _cysB topA_ Region of the F'123 Episome.** DNA was prepared from the _S. typhimurium_ strains with F'123 nonsense _supX_ mutations by the procedure of Marmur (24). From previous restriction mapping and DNA sequencing results (9, 25), it is known that the _topA cysB_ genes reside within a 5.6-kilobase region bounded by a _SpI_ restriction site on the _topA_ side and a _Sal I_ site on the _cysB_ side. Therefore, in the cloning work, several micrograms of DNA from each _supX_ mutant was mixed with 1 μg of pBR322 DNA in a total volume of 20 μl, and the mixture was digested with _SpI_. After heat inactivation of the restriction enzyme (65°C), ATP was added to 0.5 mM final concentration, and the mixture was ligated with T4 DNA ligase. The ligase was heat-inactivated (65°C for 10 min), and NaCl was added to raise the salt concentration to 150 mM for digestion with _Sal I_. After _Sal I_ digestion the mixture was extracted with phenol, precipitated with alcohol, and resuspended in 45 μl of a 10 mM Tris-HCl, pH 8.0/0.1 mM Na2EDTA buffer. Five microliters of a 10-times-concentrated DNA ligase buffer (5 mM neutralized ATP containing 100 mM each of 2-mercaptoethanol, MgCl2, and Tris-HCl, pH 7.6) was added, and ligation with T4 DNA ligase was carried out. The mixture from each _supX_ mutant was used to transform _E. coli_ strain DM700 Δ(_supX-cysB_) and was plated on minimal agar plates to select _CysB_+ transformants.

**_E. coli–_S. typhimurium Cross-Hybridization.** Southern blot hybridization (26) was performed by method C described by Davis et al. (27).

**Maxicell Method.** The maxicell method (28) used for examining plasmid-coded proteins was the modified version (29). Cells were labeled with [35S]methionine for 1 hr at 37°C.

**RESULTS**

**Nonsense Mutations of the _E. coli supX_ Gene.** To select for mutations of the _E. coli supX_ locus and rapidly identify the nonsense mutations among them, a special strain of _S. typhimurium_, PM187, designed to serve as host for the F'123 episome bearing the _E. coli supX_ gene, was constructed by a series of steps involving mutation selection and transduction (see Fig. 1). PM187, carrying the _leu-500_ promoter mutation, a deletion of the _supX_ locus that extended into the neighboring _cysB_ gene (Δ(_supX-cysB_)), and two known amber mutations, _trpE50_ (30) and _hisC340_ (31), required tryptophan (Trp+), and histidine (His+) supplementation but not leucine (Leu+); the _leu-500_ promoter mutation was suppressed by the absence of a functioning _supX_ gene.

The episome, F'123, had been shown to carry (at a minimum) the following sequence of _E. coli_ chromosomal genes: _pyrF cysB supX trpPOLEDCBA topB oppDCBA_ (7, 18, 32). The _E. coli_ tryptophan operon on F'123 would confer a Trp+ phenotype, masking the host cell's _trpE50_ amber mutation. To eliminate the _trp_ gene, we used PM785, lacking chromosomal _opp_, _topB_, and _trp_ genes, but sensitive to inhibition by triornithine because of the _opp_+ allele (20) on the F'123. When subjected to selection for _opp_ mutant clones as described (7), some of the _trp_ gene alleles simultaneously became sensitive to chromium ion and developed a requirement for tryptophan, indicating a deletion involving the _F'123 opp_, _topB_, and _trp_ genes (7). Complementation tests identified PM814 as having all of the _trp_ structural genes deleted.

The partially deleted _F'123_ from PM814 was transferred into PM187 by conjugation to create the merodiploid strain PM801 (Fig. 1). In addition to requiring histidine (His+), _tonB_ and _tryptophan_ (Trp+) due to the two amber mutations, _PM814_ also required leucine (Leu+) because of the _leu-500_ promoter mutation on the host cell chromosome and the _E. coli supX_ allele on the F'123 episome.

To create nonsense mutations in the _F'123 supX_ gene, cultures grown from single-colony subclones of PM801 were treated with 2-aminopurine to produce base-substitution mutations, and _Leu_+ clones were selected. The _Leu_+ clones represented potential epimorphic _supX_ nonsense mutations. To identify such mutations, cells from cultures of each _Leu_+ clone were spread on minimal agar medium supplemented with leucine but lacking histidine and tryptophan. Any _His_+ _Trp_+ colonies that arose, indicating suppression of the two amber mutations, were replicilated onto minimal agar medium lacking leucine to detect any return to leucine auxotrophy. The simultaneous change of all three nutritional phenotypes (His+ _Trp_+ _Leu_+ to His+ _Trp_+ _Leu_+) indicated a response to the presence of a nonsense suppressor and provided strong evidence that the _Leu_+ phenotype of the original clone had been due to the presence of a nonsense mutation in the _E. coli supX_ gene on the _F'123_ episome.

One such His+ _Trp_+ _Leu_+ clone from each treated culture was retained to assure the independent origin of each presumed _supX_ nonsense mutation. Two of these, PM806 and PM810, were chosen for further study. From another clone, PM812, all of the _Trp_+ _His_+ colonies that arose remained _Leu_+. However, when only tryptophan independence was selected, some of the _Trp_+ _His_+ colonies became _Leu_+. This suggested that nonsense suppressor-supplied amino acids acceptable at both the _trp_ and _his_ amber mutant sites were not acceptable at the _supX_ mutant site, but some which were...
acceptable at trp but not his were acceptable at supX. Further studies described below indicate, however, that the mutant alteration of the E. coli supX allele in this strain involves more than a simple nonsense mutation. The origins of PM806, PM810, and PM812 are all depicted in Fig. 1.

The F’123 episomes bearing the supX nonsense-mutant alleles from PM806 (supX503), PM810 (supX505), and PM812 (supX506) were each transferred by conjugation into E. coli strain DM800 (11) as well as E. coli strain KV1303 Str6 which has the supE44 amber suppressor allele and the supX(cysB+topA) deletion from DM700. Both strains lacked DNA topoisomerase I activity because the chromosomal supX gene was deleted. In all three DM800 E. coli strains that received the three mutant F’123 episomes, no DNA topoisomerase I activity appeared. However, in KV1303 Str6 with the supE44 amber suppressor, the strain that had received the supX-mutant F’123 from PM812 had DNA topoisomerase I activity restored; this did not occur in those receiving the F’123 supX-mutant episomes from PM806 and PM810. Apparently, the nonsense mutation in the F’123 supX gene in PM812 was an amber mutation suppressible by supE44.

Cloning the E. coli topA cysB Regions of the supX Mutant F’123 Episomes from PM806, PM810, and PM812. After checking the three strains to confirm that they exhibited the correct Leu, Trp, and His phenotypes and carried only null supX alleles, indicated by the formation of clear phase P22 plaques on lawns of their cells (2), DNA was prepared from cells of each strain by the procedure of Marmur (24). In order to clone the 5.6-kilobase Sph I–Sal I-bounded region containing the topA and the closely linked cysB genes (9, 25), the DNA preparations from the three strains were subjected to sequential digestion and ligation with the two restriction enzymes and pBR322 DNA as described. The DNA plasmid products of the final ligations were used to transform cells of E. coli strain DM700 (ΔsupX-cysB) and CysB+ transformants selected.

CysB+ transformants bearing plasmids with the presump- tive Sph I–Sal I DNA fragments, which include the cysB topA DNA region, were obtained. The plasmid pJW80 is a control obtained by cloning the Sph I–Sal I fragment of DNA from E. coli K-12 with a wild-type supX allele. The plasmids pJW83, pJW87, and pJW89 were constructed from strains PM806, PM810, and PM812, respectively; strain genotypes are shown in Fig. 1. In a separate cloning experiment, two additional CysB+ isolates transformed with PM812 DNA provided plasmids designated pJW89VI and pJW89VII.

The Clones Constructed from the DNA of the F’123 Episomes with supX Nonsense Mutations Do Contain the topA cysB Region from E. coli. Fig. 2 shows the electrophoretic patterns of HindII digests of pJW80, pJW83, pJW87, and pJW89 DNA with a lane of marker DNA fragments (lane M). The patterns of the control pJW80 plasmid and those of pJW83 and pJW87 are clearly identical and are those expected from the restriction sites known from previous restriction mapping (9) and sequencing results (25) with the cysB topA region of the E. coli chromosome. The sizes of the expected fragments are 3256, 2844, 1740, 1150, 528, and 352 bp. The patterns observed are in complete agreement with these figures except that the two largest fragments on this gel are not yet resolved and show as a thick single band at the top. Further restriction analyses with EcoRI, EcoRII, Sph I, Pvu I, Pvu I/BamHI, and BamHI/Pvu II (data not shown) confirmed the identity of the cloned DNA from PM806 (pJW83) and PM810 (pJW87) with that of the cysB topA region of the E. coli K-12 chromosome on pJW80 and the agreement with the restriction and sequencing analyses of previous studies (9, 25).

The HindII digest of pJW89 produced a quite different electrophoretic pattern (Fig. 2, lane 4). One member of the two largest fragments (not resolved in lanes 1–3: pJW80, pJW83, and pJW87) is significantly lengthened and migrated more slowly in the gel. The 1150-bp band seen in plasmid DNA samples of Fig. 2, lanes 1–3, is shortened by about 50 bp (and, therefore, slightly faster migrating) in the pJW89 digest, which also exhibits an extra band with a mobility expected of a 230-bp fragment. The independently cloned pJW89VII shows the same HindII cleavage pattern (not shown) as pJW89. The pattern (not shown) of the other independent isolate, pJW89VI, differs in that the extra 230-bp
The Products of pJW83, pJW87, and pJW89 Exhibit Little DNA Topoisomerase I Activity. In one set of experiments, strains of DM700 Δ(supX-cysB) transformed with the various plasmids were grown, pelleted, resuspended, and lysed by lysozyme treatment followed by three cycles of freezing and thawing, and the lysates then were assayed for topoisomerase I activity (10). Relaxation of the input supercoiled DNA substrate was detected only with lysates from cells with the control TopA+ plasmid, pJW80 (results not shown). In a second set of experiments, cells were lysed by treatment with lysozyme and the detergent Brij (34, 35), and the lysates were similarly assayed (gel not shown). When normalized with respect to the activity in transformants of the control plasmid, pJW80, as 100%, the levels of DNA topoisomerase I activity in transformants with pJW83, pJW87, and pJW89 are approximately 1%, 2%, and 0.1%, respectively.

The Cloned Plasmids Code for Proteins That Carry Antigenic Determinants of E. coli DNA Topoisomerase I. Fig. 3 depicts an autoradiogram of radioimmunostained E. coli DM700 Δ(supX-cysB) colonies that had been transformed with the various plasmids described above. Colonies of the transformants were first grown on a plate of LB medium containing cysteine and lysed with chloroform. A plastic sheet coated with rabbit antibodies raised against E. coli DNA topoisomerase I was then laid over the colonies to permit the binding of antigen, and the bound antigen was subsequently revealed on the autoradiogram due to the binding of 125I-labeled antibodies (36). The uppermost single spot (far left column) and the top row of four spots correspond to colonies of cells transformed with pJW83. The next three rows in descending order correspond to cells transformed with pJW87, pJW89, and pJW80, respectively. Antigenic determinants that were clearly recognized by the antibodies are present in all these transformants. Below the row of pJW80 transformant colonies was a row of colonies of transformants with pBR322. As expected, these cells contain no antigenic determinants of DNA topoisomerase I and are not associated with the radiolabeled antibodies, therefore it is conceivable that these plasmids may contain inserts of Salmonella origin.

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**Fig. 2.** The electrophoretic patterns in an agarose gel of HindII digests of DNA of pJW80 (lane 1), pJW83 (lane 2), pJW87 (lane 3), and pJW89 (lane 4). Lane M represents a mixture of restriction fragments of DNA of known length to serve as markers.

band is absent and a 1150-bp band identical to that of pJW80, pJW83, and pJW87 replaces the slightly shortened version of that fragment seen in the pJW89 and pJW89VII patterns. Further restriction mapping with the other enzymes mentioned above demonstrated that pJW89V1 differs from the control plasmid, pJW80, by the insertion of about 1400 bp within a 536-bp Pvu I digest fragment. pJW89 and pJW89VII involve more complex rearrangement in addition to that insertion.

It is interesting to note that the DNA sequence modifications and rearrangements indicated for the clones (pJW89, pJW89V1, and pJW89VII) obtained from PM812 do not themselves prevent the formation of a functional enzyme, since nonsense suppressors restore enzyme activity in both S. typhimurium and E. coli cells carrying the mutant allele(s); a nonsense codon seems to be the critical factor in preventing formation of the active enzyme. The evidence for more than one type of sequence rearrangement and the relationship to the nonsense mutation in these clones was not pursued further.

To rule out the possibility that the plasmids contain DNA derived from the topA cysB region of S. typhimurium, DNA from a wild-type S. typhimurium strain was prepared and digested with several restriction enzymes. After agarose gel electrophoresis, Southern blots were made and hybridized with a labeled plasmid containing the E. coli topA gene. Under the more stringent hybridization conditions (65°C) that give strong signals with digests of E. coli DNA, there is little cross-hybridization between the E. coli topA probe and the Salmonella DNA. When the temperature of hybridization was lowered by 15°C, faint bands were revealed on the blots of S. typhimurium DNA digests. The restriction sites deduced from these bands are markedly different from those mapped in the E. coli topA cysB region, however. Thus the topA cysB segments in pJW83 and pJW87 are clearly derived from E. coli; the same is true for pJW89 and pJW89V1.
producing no spots. This result with the row of DM700 colonies transformed with pBR322 indicates that the Δ(supX-cysB) deletion mutation on the DM700 chromosome results in either a total absence of the E. coli DNA topoisomerase I protein or the production of a peptide fragment that does not provide antigens capable of interacting with the antibodies. The spots signaling antibody–antigen interactions were, of course, expected from the colonies of cells bearing pJW80, which produce an intact and functional DNA topoisomerase I enzyme.

Various complex models utilizing supX as a positive regulator gene could be devised to explain the presence of DNA topoisomerase I antigenic determinants and the absence of enzyme activity in cells carrying pJW83, pJW87, and pJW89. However, the simple, straightforward, and reasonable interpretation is that the supX gene is topA, the structural gene for the enzyme, and the supX nonsense mutations in pJW83, pJW87, and pJW89 created termination codons at positions that permit the synthesis of fragments of the enzyme large enough to provide antigenic determinants but not enzyme activity.

The Results of the Use of the Maxicell Technique. Use of the maxicell method to examine, in an electrophoresed gel (not shown), the plasmid-coded proteins from DM700 cells transformed with the control TopA+ plasmid revealed the 100-kDa protein band expected from the presence of an intact DNA topoisomerase I protein. On the gel in the lanes representing the maxicell lysates with the plasmid-coded proteins of the three DM700 clones transformed with plasmids bearing the supX nonsense mutations, the 100-kDa protein band was missing. However, each had one new protein: a 47-kDa protein in the transformant with pJW83, a 36-kDa protein in the transformant with pJW87, and a 64-kDa protein in the transformant with pJW89. It seems reasonable to interpret these new bands as the peptide fragments resulting from terminations at the nonsense codons in the gene that codes for the 100-kDa DNA topoisomerase I protein.

**DISCUSSION**

The results described here demonstrate that several independent nonsense mutations of the E. coli supX locus all lead to the disappearance of the intact DNA topoisomerase I protein. In each case it is replaced by a smaller peptide, differing in size for each mutation, but all carrying antigenic determinants specific to the DNA topoisomerase I enzyme. This seems to be firm proof that E. coli supX mutations, selected by their suppression of the leu-500 promoter mutation in an S. typhimurium host cell, are located in the structural gene for the enzyme and validates the use of the gene designation topA rather than supX. Therefore, the mutant allele designations supX503, supX505, and supX506 shown in Fig. 1 for strains PM806, PM810, and PM812, respectively, should be changed to topA503, topA505, and topA506.

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