Mutations in the gene coding for Escherichia coli DNA topoisomerase I affect transcription and transposition
(ω protein/DNA supercoiling/supX)

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ABSTRACT Mutations in top, the structural gene for Escherichia coli DNA topoisomerase I, have been identified and mapped at 25 min on the chromosome, near cysB. Strains carrying deletions of the top gene are viable. The top mutations, however, do exert pleiotropic effects on transcription and transposition. Mutants lacking DNA topoisomerase I have a more rapid rate of induction and a higher level of catabolite-sensitive enzymes including tryptophanase and β-galactosidase. This general activation of transcription by top mutations can be attributed to an increase in the negative superhelicity of the DNA in vivo when the topoisomerase activity is abolished. The frequency of transposition of Tn5, a transposon carrying kanamycin resistance, is decreased by a factor of 40 or more in top mutants. A direct or indirect role of the topoisomerase in transposition is discussed. The transposition frequency of Tn3, however, is not dependent on top. Based on the studies of the E. coli top mutants, it appears that the supX gene, which was originally studied in Salmonella typhimurium [Dubnau, E. & Margolin, F. (1972) Mol. Gen. Genet. 117, 91–112] is likely to be the structural gene for DNA topoisomerase I.

Escherichia coli DNA topoisomerase I, also known as ω protein, has been the subject of extensive studies (for a review, see ref. 1). The enzyme is a single polypeptide of about 110,000 daltons. In vitro it catalyzes the relaxation of negatively supercoiled DNA (2), the knotting and unknotting of single-stranded DNA rings (3), the linking of single-stranded DNA rings of complementary sequences into intertwined duplex rings (4), and the catenation and decatenation of duplex DNA rings when at least one member of a pair of participating duplex rings has a single-chain scission (5). The catalysis of these topological isomerization reactions by the enzyme is believed to involve transient single-stranded breakage of DNA phosphodiester bonds (1).

In contrast to the extensive in vitro studies, there has been little work on the functions of the enzyme in vivo. We have therefore undertaken the isolation and characterization of mutants deficient in DNA topoisomerase I activity. In this communication, we report the identification of a number of mutations in the structural gene for the enzyme and the genetic mapping of this gene, which we have termed top, on the E. coli chromosome. Roles of the enzyme in the regulation of transcription of a number of operons and in the transposition of several transposons are also implicated based on our initial characterization of the mutants.

MATERIALS AND METHODS

Bacterial Strains. All strains used were E. coli K-12 derivatives. The strain used to produce the collection of temperature

dissociation sensitive mutants was PA3092 F' thr leu thi argH thrA his trp lacY1 mtl xyl malA mel tonA str supE. Two mutants from this collection were found to have top mutations, JE10010 top10 and JE10250 top250 (6). Other strains used include: PS49 Hfr met; KV385 met lacY1 mtl xyl str top10; PLK381 F' trpE pyrF gal-25 nirA strA195; JTT1, a trp' top' transductant of PLK381; RS2, a trp' top10 transductant of PLK381; and AB1369 F' thi-1 argE3 cysB38 proA2 his-4 galK2 lacY1 xyl-5 tss-29 supE44. From D. Mascarenhas we received the following derivatives of W3110: DM4100 cysBam242, DM600, DM700, DM750, and DM900. The last four strains are supX and cysB deletions. RL88 is described in ref. 7. The E. coli–ColE1 hybrid plasmids are in strain JA200 F' ΔtrpE5 recA thr leu lacY (8).

Bacterial Growth Conditions. Bacteria were grown in Penassay (Difco) or Luria broth (LB) in M9 minimal medium (9). All media were supplemented with filter-sterilized cysteine at 40 μg/ml for growth of cysteine auxotrophs and thymine at 10 μg/ml for growth of thymine auxotrophs. M9 medium was supplemented with either 0.5% Casamino acids or the required amino acids at 50 μg/ml. Growth of bacteria was followed by turbidity measurements in a Klett instrument. The ultraviolet light and methyl methanesulfonate sensitivities of top mutants were measured as described (10).

DNA Topoisomerase I Assay. Strains to be assayed were grown overnight in 5 ml of LB. The culture was pelleted and resuspended in 2 ml of lysis buffer [10% (wt/vol) sucrose/0.08 M potassium phosphate, pH 7.9]. Cells were repelletted, re-suspended in 0.5 ml of lysis buffer, and lysed by sonication with a Branson sonifier. The lysate was centrifuged for 15 min at an Eppendorf table-top centrifuge and the supernatant was used for assays.

In a typical assay, 2 μl of a 1:10 diluted extract was mixed with 8 μl of PM2 DNA and reaction buffer such that the final concentrations were: DNA, 20 μg/ml; potassium phosphate, 0.08 M at pH 7.9; sucrose, 8%; MgCl2, 3 mM; Na2EDTA, 1 mM; and E. coli tRNA, 100 μg/ml. Samples were incubated at 42°C for 40 min and the reaction was terminated by the addition of 2 μl of 0.2 M Na2EDTA containing bromphenol blue.

The extent of reaction was monitored by electrophoresis in 0.7% agarose gels with 0.05 M Tris, pH 8/0.05 M boric acid/1 mM Na2EDTA. Electrophoresis was for 16 hr at 4 V/cm at room temperature. Gels were stained with ethidium bromide (0.5 μg/ml), illuminated with ultraviolet light, and photographed with type 57 Polaroid film.

Tryptophanase and β-Galactosidase Assays. Cells were grown to midlogarithmic phase in M9 medium containing 0.1% glucose, 0.5% Casamino acids, and 50 μg of uracil per ml. Induction was started by the addition of tryptophan to 1 mg/ml (final concentration) or isopropyl β-thiogalactoside to 1 mM.
When cyclic AMP was used, it was added at the same time as the inducer, to a final concentration of 33 mM.

Tryptophanase was assayed in toluenized cells essentially as described (11). At each time point, 1.5-ml portions of the cell culture were chilled, concentrated 15-fold, toluenized, and assayed for enzyme. The concentration of indole was measured by adding 0.5 ml of 5% p-aminomethylbenzaldehyde in ethanol plus 2 ml of HCl/ethanol (8 ml of conc. HCl + 92 ml of ethanol) to the reaction mixture. After 30 min, the optical absorbance was measured at 575 nm. A unit of tryptophanase activity is defined as (1000 A575/As00 of the cell culture).

β-Galactosidase was assayed as described by Miller (9).

Results

Mutant Isolation. The use of a collection of E. coli mutants of independent origin for the screening of mutations in a given gene has been reported (14-18). Although isolates of this collection were originally selected for their thermal sensitivity, mutations unrelated to the thermal sensitivity are common because of the severe mutagenesis used. About 800 isolates of this collection were grown separately at 30°C, a permissive temperature, and cells from each were pelleted, washed, and lysed. Assays for DNA topoisomerase I activity in the lysates were then carried out at 42°C, a nonpermissive temperature for the growth of the cells. Details of the procedure have been described (6).

Topoisomerase I cleavage activity was assayed by the methods similar to those for the substrate and gel electrophoresis (Fig. 1A). A shift of the most rapidly migrating DNA species in the gel is fully supercoiled PM2 DNA (form I). The most rapidly moving species is unit-length linear DNA (form II). Relaxation of the supercoiled DNA is associated with a shift of its electrophoretic mobility toward that of the nicked circular form. The sample in lane c shows a family of partially relaxed DNA topoisomers migrating between the linear and nicked circular DNA.

for DNA topoisomerase I activity. Many of the temperature-resistant recombinants showed low topoisomerase activity characteristic of the original mutant, JE10010. Thus, the thermal sensitivity of JE10010 and its topoisomerase defect are unrelated and due to separate mutations. Of eight recombinants that received the trp marker from the donor, seven showed normal levels of the topoisomerase activity. None of the nine other genetic markers screened correlated with the restoration of topoisomerase activity. These results indicate that the mutation responsible for the reduction of the topoisomerase activity maps near the trp gene. Results to be shown below demonstrate that this mutation is in the structural gene for DNA topoisomerase I. This gene is denoted top and the mutant allele is present in JE10010 top10.

The refined mapping of the top gene was carried out as follows. One of the trp top10 recombinants from the above-described conjugational cross was converted to trp + by P1 transduction. The resultant strain, called KV385, was used to prepare a P1 lysate. This was used to transduce strain PLK831 trp pyrF top +. Trp +, PyrF +, and Trp +, PyrF + cotransductants were isolated and assayed for topoisomerase I activity. Of 20 Trp + transductants examined, 13 were defective in this enzyme. Similarly, 10 of 20 PyrF + transductants and 8 of 8 Trp +, PyrF + cotransductants were Top - . These data indicate that the top gene lies between trp and pyrF at about 28 min on the E. coli genetic map (24). The linkage between top and cysB, a gene that is known to be at 28 min, was then tested by using the P1 lysate of strain KV385 to transduce strain AB1369 cysB. All 35 CysB + transductants examined were Top + . AB1369 itself is Top + . These results show that the top gene is near, but distinct from, cysB. Similar experiments showed that the top250 allele present in strain JE10250 is also tightly linked to cysB.

Deletion Mutants of top. The close proximity of top and cysB led us to examine mutants known to be deleted in the cysB region. Lysates of four independently isolated cysB deletions, DM600, DM700, DM750, and DM800, were found to have no detectable topoisomerase I activity. Another strain, RL88,
which is known to be deleted for the region tonB-cysB (7), similarly showed no topoisomerase I activity in the lysate. The simplest interpretation is that in these strains part or all of the top gene is deleted. These results also demonstrate that at least the bulk of the DNA relaxation activity detected in lysates of top10 and top250 strains is due to residual activity of topoisomerase I.

top Is the Structural Gene for DNA Topoisomerase I. Several lines of evidence indicate that top is the structural gene for the topoisomerase. First, proteins from lysates of top1, DM700 (a top deletion), top10, and top250 strains were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis followed by transfer of the protein bands to a nitrocellulose sheet and staining of the bands with 125I-labeled rabbit antibodies against E. coli DNA topoisomerase I. No band was detectable on the autoradiogram with lysate from DM700, in agreement with the view that DM700 is deleted in top. The patterns with lysate from top1 and top250 were essentially identical, with a prominent band corresponding to a polypeptide of 110,000 daltons and several fainter bands of higher electrophoretic mobilities that probably represent proteolytic products. Lysate from top10 showed no 110,000-dalton band. Instead, a prominent band of 70,000 daltons was seen, indicating that top10 is likely to be a chain-termination mutation.

Further evidence supporting the notion that top is the structural gene came from a screening of the Clarke–Carbon library of ColEl plasmids carrying segments of E. coli DNA (8). None of the six plasmids known to carry trpE, three were found to carry cyaB. These three, pLC5-23, 4-6, and 41-15, were mated with DM800, one of the top cyaB deletion strains described in the previous section, and recombinants that are cyaB+ and ColE1-resistant were selected and assayed for DNA topoisomerase I activity. It was found that recombinants carrying pLC5-23 or 4-6 have acquired the topoisomerase activity, whereas those carrying pLC41-15 have not. DM800 strains harboring pLC5-23 and pLC41-15 were then analyzed for proteins that are coded for by the plasmids, by using the maxi-cell technique (25). pLC5-23, but not pLC41-15, produced a 110,000-dalton protein that comigrated with authentic DNA topoisomerase I on a dodecyl sulfate/polyacrylamide gel (data not shown).

Properties of the Mutants. The existence of top deletion mutants shows that the gene is not essential for the viability of the bacterium. DM800(topΔ) grows with a doubling time 10–20% longer than that of comparable top1 strain, DM4100. In addition to the strains deleted in top, two phage P1 transductants generated during the mapping experiments, JTT1(top) and RS2(top10), were also used to investigate the mutant phenotype. This pair of transductants is isogenic and contains less than 0.8% of the heavily mutated chromosome present in the original top10 mutant, JE10010. JTT1 and RS2 have the same growth rate in both broth and minimal medium at temperatures ranging from 30°C to 42°C. Both plate bacteriophages λ, T7, and T4 with normal efficiency. Phage Ac587 can lysogenize a top10 strain and can be induced normally from it. Phage mu plates poorly on top mutants.

The genetic experiments used to map the top mutation showed that strains carrying the top10 mutation can participate as a recipient in conjugation or P1 transduction. The same is true for DM800(topΔ). Other experiments have shown that a top10 strain can also be used as a donor in conjugation. In all cases, the observed recombination frequency was normal.

Both the top10 and topΔ mutants were somewhat sensitive to ultraviolet light. At a dose of about 104 ergs/mm2, 0.1% of DM4100(top10) cells survived but only 0.001% of DM800(topΔ) survived. A top10 strain also was sensitive to ultraviolet but less so than DM800(topΔ). The ultraviolet sensitivity observed for top strains was comparable to that seen for E. coli DNA ligase mutants (10) but far less than that seen for recA mutants.

The top mutants also were sensitive to methyl methanesulfonate. The plating efficiency of DM800(Dtop) was less than 4 × 10−3 compared to DM4100(top10) on plates containing methyl methanesulfonate. Under the same conditions, a top10 mutant had a normal plating efficiency but the colonies were much smaller than for a comparable top1 strain.

Mutations in top Affect the Expression of Certain Operons. There have been several reports that transcription from certain promoters, including those controlling catabolite-sensitive operons, is sensitive to drugs that inhibit DNA gyrase (26–30). The inference that can be made from such studies is that transcription from some promoters, but not all, is dependent on the superhelicity of the DNA (27–30). Because in vivo the superhelicity of a covalently closed DNA is affected by both DNA gyrase and topoisomerase I, it appears plausible that top mutations can also influence the expression of certain operons. We therefore examined several systems. In the case of the tryptophanase gene, the induction of enzyme activity was more rapid and went to a higher level in the top1 mutant compared to an isogenic top1 strain (Fig. 2A). In the example shown, cells were grown in medium containing 0.1% glucose, a condition in which there is some catabolite repression. Addition of cyclic AMP to this medium (Fig. 2A) or growth in a glycerol medium, both of which alleviate catabolite repression, decreased the difference observed between the two strains. Similar results were obtained for the induction of β-galactosidase in 0.1% glucose medium (Fig. 2B).

![Fig. 2 Induction of tryptophanase and β-galactosidase activity in JTT1(top) and RS2(top10). (A) Tryptophanase activity without (Left) and with (Right) cyclic AMP. (B) β-Galactosidase activity without (Left) and with (Right) cyclic AMP. For the tryptophanase experiment, the 0-min sample was taken immediately after the addition of inducer. Therefore, differences in the basal levels of tryptophanase are probably not significant.](image-url)
The level of two enzymes involved in the biosynthesis of isoleucine and valine was measured. One enzyme, acetohydroxy acid synthase from the ilB gene, is under cyclic AMP control (31), the other enzyme, threonine deaminase, is not. The basal level of the ilB enzyme under repressed conditions is three times higher in the top mutant than in the top⁻ strain, whereas the level of threonine deaminase is the same in the two strains under the same conditions (M. Freundlich, personal communication).

**top** Mutation Decreases Transposition of Tn5 but not Tn3.

The transposition frequency of Tn5, a transposon that carries kanamycin resistance, was strongly affected by the top mutation (Table 1). In RS2(top10), the frequency was decreased by a factor of 40 and in the Δtop mutants it was even lower. The frequency of transposition of transposons Tn9 and Tn10 is also much decreased in these mutants (N. Kleckner and D. Roberts, personal communication). In contrast, the frequency of transposition of the ampicillin-resistance-carrying transposon Tn3 is unaffected in top mutants. We found a normal frequency of about $8 \times 10^{-6}$ for Tn3 transposition in either top⁻ or top10 strains.

### DISCUSSION

A number of reports have demonstrated that the levels of expression of certain genes, including several catalytically regulated operons and the rRNA genes, are decreased by the inhibition of DNA gyrase (26–30) or by the addition of intercalating dyes (32). We show here that the levels of expression of several genes are increased by mutations in the gene coding for DNA topoisomerase I. Because in vitro the two enzymes are known to have diametrically opposing effects with regard to the supercoiling of a covalently closed double-stranded circular DNA, the picture that evolves from the two sets of studies is that the superhelicity of the bacterial chromosome in vivo is determined by the combined actions of at least two, and perhaps more, topoisomerases. Inhibition of DNA gyrase tips this dynamic balance toward a lower negative superhelicity (33), whereas abolishing DNA topoisomerase I is expected to have the opposite effect. The latter expectation is supported by preliminary measurements of the superhelicity of plasmid DNAs isolated from top mutants (unpublished data). In vitro studies of the effects of superhelicity on the transcription of several promoters by purified RNA polymerase show that in general an increase in the negative superhelicity of the DNA enhances transcription (34–37). For the wild-type lac promoter under the positive regulation of the cyclic AMP receptor protein, CRP, recent in vitro studies show that specific transcription is enhanced by both the receptor and negative superhelicity (W. McClure and H. Buc, personal communication). Thus, the observed effects of the topoisomerases on transcription in vivo are entirely consistent with these in vitro results. The possibility of a general regulatory mechanism involving the superhelicity of the chromosome has been raised previously (35); our present studies point to the interesting question of how the topoisomerase genes are regulated.

The strong effect of the top mutation on transposition frequencies of several transposons can be interpreted in terms of either a direct role of the topoisomerase in these transpositions or an indirect effect. The in vitro catalytic property of the enzyme suggests, in analogy to the phage $\lambda$ int topoisomerase (38), that the top product might participate directly in the breakage and rejoining of DNA strands during transposition. Alternatively, topoisomerase I could be involved in the intertwining of strands between DNA molecules which might be required prior to transposition. In the case of Tn3, such a catalytic or DNA-alignment role of the top product would have to be fulfilled by a transposon-encoded enzyme or by a different host topoisomerase. On the other hand, the effects of top mutations on transposition might be completely indirect. For example, a transposition event might require the product of a gene that is under the negative control of a repressor: top mutations increase the transcription of the repressor gene which in turn shuts off the transposition determinant.

The effects of top mutations on transcription and the close linkage of the top gene to cryB strongly suggest that top might be identical to a gene supX that has been studied extensively in *Salmonella typhimurium* (39–41). Mutations of the supX locus of *S. typhimurium* exhibit pleiotropic effects including suppression of several promoter mutations and increase of alkaline phosphatase levels (40, 41). Recent studies show that the chromosomal locations of the supX loci of *E. coli* and *S. typhimurium* are identical (42). Further support for the identity of top and supX comes from our observation that *S. typhimurium* carrying a supX amber mutation exhibits no antigenic determinants that are recognized by rabbit antibodies raised against *E. coli* DNA topoisomerase I (unpublished data). On the other hand, the levels of antigenic determinants are normal for the same supX mutation in an isogenic strain carrying an amber suppressor. We have shown previously that rabbit antibodies raised against *E. coli* DNA topoisomerase I are equally efficient in inhibiting the *S. typhimurium* enzyme (unpublished results described in ref. 1). These observations do not rule out the possibility, however, that supX and top are distinct genes but that a functional product of the former is required for the production of the latter.

The viability of cells deleted in the top region shows that DNA topoisomerase I is not essential for DNA replication. The topological constraint for strand separation, especially when the complementary strands are in the form of closed rings, apparently can be alleviated by other enzyme systems such as DNA gyrase or other topoisomerases yet to be identified. The role of gyrase in replication is well established (43). It is not known whether there are other DNA topoisomerases that are required in addition to gyrase or in place of DNA topoisomerase I. The availability of top mutations makes it more convenient to search for such enzymes.

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**Table 1.** Tn5 transposition in top mutants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Transposition frequency</th>
<th>Ratio, top⁻/top⁺</th>
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<tr>
<td>JTT1</td>
<td>top⁻</td>
<td>$4.7 \times 10^{-6}$</td>
<td></td>
</tr>
<tr>
<td>RS2</td>
<td>top10</td>
<td>$1.2 \times 10^{-5}$</td>
<td>40</td>
</tr>
<tr>
<td>DM2100</td>
<td>top⁻</td>
<td>$1.6 \times 10^{-4}$</td>
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<tr>
<td>DM580</td>
<td>Δtop</td>
<td>$1.8 \times 10^{-6}$</td>
<td>90</td>
</tr>
<tr>
<td>DM700</td>
<td>Δtop</td>
<td>$2.2 \times 10^{-6}$</td>
<td>70</td>
</tr>
</tbody>
</table>
12. Berg, D. (1977) in *DNA Insertion Elements, Plasmids, and Epi-

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