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Identification and localization of a gene that specifies production of *Escherichia coli* DNA topoisomerase I

(ω protein/radioimmunoassay/F′ plasmid/*Salmonella typhimurium*)

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ABSTRACT **A gene that specifies production of *Escherichia coli* DNA topoisomerase I (ω protein) was identified with the aid of a radioimmunoassay for this protein. *E. coli* DNA topoisomerase I was produced by *Salmonella typhimurium* merodiploids that harbored *E. coli* plasmid F′ 123, but not by strains that lost this plasmid. Analysis of strains with spontaneous deletions of F′ 123 showed that the gene, *topA*, required for production of the *E. coli* ω protein was between the *trp* operon and the *cysB* gene. Deletions that eliminated *topA* also eliminated the *supX* gene. We suggest that *topA* is the structural gene of *E. coli* DNA topoisomerase I and that *topA* is identical to *supX*.**

DNA topoisomerases are ubiquitous enzymes that catalyze the interconversion of DNA topological isomers by introducing transient breaks into DNA chains (1–3). *Escherichia coli* DNA topoisomerase I (ω protein) (4) is the archetype of prokaryotic DNA topoisomerases that break and rejoin one DNA strand at a time [type I topoisomerases according to the nomenclature of Liu *et al.* (5)]. In the absence of high-energy cofactors, this enzyme catalyzes several reactions *in vitro*, including the relaxation of supercoiled DNA (4), the knotting and unknotting of single-stranded DNA rings (6), and the intertwining of single-stranded rings of complementary sequences (7).

Identifying and locating the structural gene of DNA topoisomerase I is basic to any understanding of its physiological role. We have approached this problem in the absence of mutants by utilizing the natural differences between *E. coli* DNA topoisomerase I and the homologous enzyme of *Salmonella typhimurium*. Similar techniques have been used to map several other genes (8–11). We report here the identification and location of an *E. coli* gene that is required for the production of *E. coli* DNA topoisomerase I in merodiploid strains of *S. typhimurium*.

MATERIALS AND METHODS

Bacterial Strains. The strains of *E. coli* K-12 and *S. typhimurium* LT2 used are described in Table 1. The presence of F′ plasmids was verified by curing in the presence of acridine orange with concomitant acquisition of appropriate auxotrophic requirements or by conjugation (16). Transductions mediated by phage P22 were performed as described (12).

Enzymes. *E. coli* DNA topoisomerase I was purified from strain RY13 as described (17). *S. typhimurium* DNA topoisomerase I was purified from strain RED10 by a similar procedure. Both enzyme preparations were at least 90% homogeneous on sodium dodecyl sulfate/polyacrylamide gels.

Immunochemical Reagents. Rabbit antiserum 3271-1214 was prepared against *E. coli* DNA topoisomerase I at the Pocono Rabbit Farm (Canadensis, PA). The antiserum was incubated at 50°C for 20 min and was used without further purification. *Staphylococcus aureus* immunoadsorbent (IgGsorb) was from The Enzyme Center (Boston, MA).

Radiolabeled Antigen. *E. coli* DNA topoisomerase I was iodinated by the procedure of Fraker and Speck (18). The reaction was initiated by addition of approximately 1 mCi (3.7×10^7 becquerels) of $Na^{125}I$ (IMS-30, Amersham) to 11 μg of the topoisomerase in 90 μl of 150 mM NaCl/50 mM sodium phosphate, pH 7.4, in a reaction vessel that contained 10 μg of 1,3,4,6-tetrachloro-3,6a-diphenylglycouril (Iodo-Gen, Pierce). The reaction was terminated after 10 min at room temperature by addition of sodium metabisulfite (100 μg in 10 μl), and the radiolabeled antigen was isolated by exclusion chromatography through a 8-ml column of Bio-Gel P-6 (Bio-Rad) equilibrated with 150 mM NaCl/50 mM sodium phosphate, pH 7.4/0.1% bovine serum albumin/0.05% Triton X-100 (assay buffer). Radiolabeled topoisomerase prepared by this method retained the molecular weight of the unlabeled enzyme and had a useful life of approximately 4 weeks.

Radioimmunoassay. Extracts of strains to be assayed were prepared from 35 ml of stationary-phase cultures as described (12). Protein contents of extracts were determined by the method of Lowry *et al.* (19). Dilutions of radiolabeled antigen and antiserum were made in assay buffer and centrifuged at 15,000 rpm for 5 min in an Eppendorf model 5412 microcentrifuge before use in the assay.

Assays were performed in triplicate in 400-μl polyethylene microcentrifuge tubes as follows: One hundred microliters of diluted radiolabeled antigen (20,000 cpm) and 50 μl of competitive antigen were mixed in each tube; 50 μl of antiserum 3271-1214, diluted 1:400, was added and mixed with the antigens. After 30 min at room temperature, 50 μl of a 2% suspension of IgGsorb was added and mixed with the antigen/antiserum mixture. After at least 10 min, each tube was centrifuged for 1 min in the microcentrifuge at room temperature. From each assay tube (*A*) one-half (125 μl) of the supernatant was transferred to another tube (*B*). Radioactivity in each tube was measured in a Packard Auto-Gamma scintillation spectrometer. The fraction of bound antigen in each assay tube was determined by the formula:

$$F = \text{fraction bound} = \frac{A\ (\text{cpm}) - B\ (\text{cpm})}{A\ (\text{cpm}) + B\ (\text{cpm}) - 2 \times \text{background (cpm)}}.$$

In the absence of competitive antigen, the fraction of bound antigen, $F(0)$, ranged between 50% and 30% for the assays reported herein. Percent competition in the presence of com-

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Abbreviations: Trp^-, Cys^-, and Ura^-, auxotrophic requirements for tryptophan, cysteine, and uracil, respectively.

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Table 1. Bacterial strains

Strain	Genotype or phenotype	Origin
Salmonella typhimurium LT2		
RED2	Wild type	Ref. 12
RED10	*endA41*::Tn*10*	Ref. 12
MT10	*trpE52 cysB12 pyrF146*	F⁻ segregant of TR5352
MT100	*trpE52 cysB12 pyrF146 endA41*::Tn*10*	RED10(P22) × MT10
MT101	*cysB12 pyrF146 endA41*::Tn*10*	RED2(P22) × MT100
MT102	*pyrF146 endA41*::Tn*10*	RED2(P22) × MT100
PM214	*supX13* (deletion into *cysB*) *leu-500 ara-9*	P. Margolin (13)
Escherichia coli K-12		
RY13		P. Modrich (14)
E. coli F′/*S. typhimurium* merodiploids		
SB3073	F′142/*guaA1 his-3217*	P. E. Hartman (15)
SB3184	F′14/*metB36 purG302*	P. E. Hartman (15)
TM7	F′150/*hisF645*	J. Heitig (11)
TR132	F′*pro*⁺ *lac*⁺/*adeC7 proA46 str*ᴿ *ile405 gal501*	J. Roth
TR1359	F′116/*serA790 lys-554*	J. Heitig (11)
TR5347	F′133/*hisD2421 metE338 ilvC401 ara-9*	J. Roth
TR5349	F′254/*trpA8 purE801*	J. Roth
TR5350	F′152/*hisO1242 hisB2142 nic-506 hut*⁺ *galE542*	J. Roth
TR5352	F′123/*trpE52 cysB12 pyrF146*	J. Roth
TR5358	F′254/*purE66 gpt-3*	J. Roth
TR5360	F′150/*serA790 lys-554 his64*	J. Roth
TT1945	F′117*zzf-20*::Tn*10*/*pyrB64 mel hisD6414*	J. Roth
TT1946	F′129*zzf-20*::Tn*10*/*hisO1242 hisC3737 aroD5 purF145 glpT*	J. Roth
TT1948	F′152 *zzf-20*::Tn*10*/*hisO1242 hisB2142 nic-506 hut*⁺ *galE542*	J. Roth
Derivatives of TR5352 with mutations in F′123:		
MT2007-MT2502	Trp⁻ Cys⁻	This work
MT3008-MT3708	Cys⁻ Ura⁻	This work
MT4204-MT4410	Trp⁻	This work
MT5005-MT5801	Cys⁻	This work

(P22) indicates source of transducing lysate. Trp⁻, Cys⁻, and Ura⁻ indicate requirements for tryptophan, cysteine, and uracil, respectively.

petitive antigen was derived from the bound fraction, $F(c)$, by the formula:

$$\text{competition (\%)} = 100 - \frac{F(c) \times 100}{F(0)}.$$

Bacterial extracts were diluted in assay buffer, if necessary, to give 40% to 70% competition.

RESULTS

***E. coli* and *S. typhimurium* ω Proteins Can Be Distinguished by Radioimmunoassay.** Purified ω proteins from *E. coli* or *S. typhimurium* differed in their abilities to compete with the radiolabeled probe (*E. coli* DNA topoisomerase I) in the radioimmunoassay that we developed. Fig. 1 presents data from experiments that used these two enzymes as competitive antigens. As shown, *E. coli* DNA topoisomerase I gave a higher percent competition than an equal amount of *S. typhimurium* DNA topoisomerase I. The differences in the slopes and apparent maxima of the competition curves are also characteristics that can be used to distinguish these proteins.

The radioimmunoassay was used to measure the percent competition due to competitive antigen in extracts of various strains of *E. coli* and *S. typhimurium* (see below). The amount of purified *E. coli* DNA topoisomerase I that would give the same competition was determined from the *E. coli* competition curve shown in Fig. 1. This procedure was designed to estimate the amount of *E. coli* ω protein in the presence or absence of *S. typhimurium* ω protein. Experiments with extracts of haploid strains of *E. coli* and *S. typhimurium* indicated that the major competitive antigens in the extracts had the characteristics of the corresponding purified ω proteins.

Identification of a *S. typhimurium* Merodiploid Containing Highly Competitive Antigen. A search for a structural gene for *E. coli* DNA topoisomerase I was carried out by determining the apparent amount of *E. coli* ω protein present in extracts of *S. typhimurium* merodiploid strains harboring *E. coli* F′ plasmids. We expected that a merodiploid strain carrying this gene would contain both *E. coli* and *S. typhimurium* ω proteins, giving rise to considerably higher competition and thus higher apparent *E. coli* ω protein content, provided that the *E. coli* structural gene could be expressed in *S. typhimurium*. The results

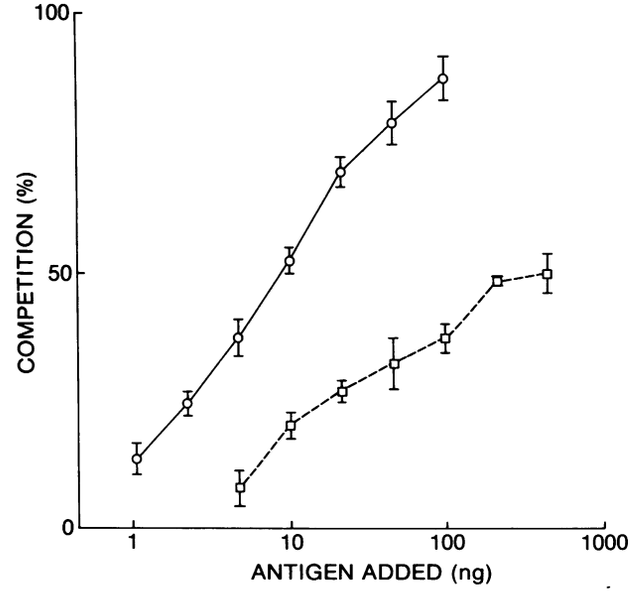

FIG. 1. Competition curves for purified *E. coli* and *S. typhimurium* ω proteins. The indicated amounts of these proteins were added as competitive antigens in the radioimmunoassay and the resulting percent competition was determined. ○, *E. coli* DNA topoisomerase I, average and standard deviations for six determinations from two independent experiments. □, *S. typhimurium* DNA topoisomerase I, average and standard deviations for three determinations.

Table 2. Apparent *E. coli* DNA topoisomerase I antigen in extracts of *E. coli* and *S. typhimurium*

Strain	*E. coli* F′ plasmid	Antigen/total protein, ng/mg
S. typhimurium merodiploid strains		
SB3073	F′142	46
SB3184	F′14	40
TM7	F′150	42
TR132	F′pro lac$^+$	86
TR1359	F′116	51
TR5347	F′133	45
TR5349	F′254	49
TR5350	F′152	58
TR5352	F′123	520
TR5358	F′254	64
TR5360	F′150	67
TT1945	F′117 *zzf-20*::Tn*10*	35
TT1946	F′129 *zzf-20*::Tn*10*	69
TT1948	F′152 *zzf-20*::Tn*10*	55
E. coli		
RY13	None	400

of determinations on 14 different merodiploid strains of *S. typhimurium* are presented in Table 2. One of these strains, TR5352, contained competitive antigen equivalent to even more *E. coli* DNA topoisomerase I than was found in extracts of haploid *E. coli* strains. In contrast, the average apparent *E. coli* ω protein content of the 13 other merodiploid strains was 10% of the TR5352 value, suggesting that these strains do not contain the *E. coli* enzyme.

The competitive antigen present in extracts of strain TR5352 was identical to *E. coli* DNA topoisomerase I by two criteria: undiluted extracts gave greater than 90% competition in the radioimmunoassay; and determinations of percent competition versus dilution of TR5352 extracts (data not shown) paralleled the competition curve for the authentic *E. coli* protein shown in Fig. 1. Both of these criteria were met by extracts of *E. coli* strains, but neither was met by the other *S. typhimurium* extracts or by purified *S. typhimurium* DNA topoisomerase I.

We interpret these data to mean that strain TR5352 contains *E. coli* DNA topoisomerase I, and we infer that this *S. typhimurium* merodiploid strain contains a structural gene for this *E. coli* enzyme. We propose that this gene be designated *E. coli topA*.

E. coli topA Is Located Between the _trp_ Operon and _cysB_ on F′123. To determine the location of the *topA* gene, derivatives of strain TR5352 that had lost one or more episomal markers were tested by radioimmunoassay for presence or absence of *E. coli* DNA topoisomerase I. Strain TR5352 is prototrophic because its chromosomal mutant alleles are complemented by the corresponding genes in the *E. coli* plasmid F′123. An F$^-$ segregant of TR5352, such as strain MT10, displays auxotrophic requirements for tryptophan, cysteine, and uracil. Spontaneous auxotrophic derivatives of TR5352 were isolated after ampicillin enrichment (16). Strains with two auxotrophic requirements were more frequent than strains with single requirements. From five independent ampicillin enrichments we recovered 289 Trp$^-$ Cys$^-$ and 340 Cys$^-$ Ura$^-$ derivatives, but only 35 Cys$^-$ and 5 Trp$^-$ strains.

Results of our analyses of representative strains from these different phenotypic classes are presented in Table 3. Loss of the entire F′ plasmid resulted in loss of the *E. coli* ω protein (compare TR5352 and MT10). Of 18 Trp$^-$ Cys$^-$ derivatives tested, none contained *E. coli* DNA topoisomerase I. Of 14 Cys$^-$ Ura$^-$ strains tested, 9 did not contain *E. coli* ω protein,

Table 3. Influence of F′123 mutations on apparent *E. coli* DNA topoisomerase I antigen

Strain	Phenotype*				Antigen/total protein, ng/mg
	Trp	Top	Cys	Ura	
TR5352	+	+	+	+	590
MT10	−	−	−	−	64
MT2101	−	−	−	+	72
MT2202	−	−	−	+	37
MT2305	−	−	−	+	59
MT2502	−	−	−	+	44
MT3301	+	−	−	−	47
MT3404	+	−	−	−	61
MT3502	+	−	−	−	36
MT3601	+	−	−	−	36
MT3008	+	+	−	−	780
MT3401	+	+	−	−	920
MT3405	+	+	−	−	920
MT3406	+	+	−	−	710
MT4204	−	−	+	+	68
MT4216	−	−	+	+	87
MT4238	−	−	+	+	100
MT4335	−	−	+	+	69
MT4410	−	+	+	+	780
MT5012	+	−	−	+	21
MT5121	+	−	−	+	36
MT5207	+	−	−	+	44
MT5402	+	−	−	+	22
MT5526	+	+	−	+	900
MT5709	+	+	−	+	720
MT5735	+	+	−	+	700
MT5801	+	+	−	+	1000

* Top, *E. coli* DNA topoisomerase I; −, auxotrophic requirement or absence of antigen; +, ability to make product.

but 5, did have this antigen. Four of the Trp$^-$ strains had lost the *E. coli* protein and 1 had not. Of 28 Cys$^-$ strains assayed for the *E. coli* antigen, 14 were negative and 13 were positive. One Cys$^-$ strain, MT5601, repeatedly gave intermediate values in the range of 250 ng of apparent *E. coli* ω protein per mg of total protein from independent extracts.

We interpreted the simultaneous loss of two or more closely linked episomal genes as resulting from a single deletion event. Loss of *E. coli* DNA topoisomerase I antigen was interpreted as inactivation of the *topA* gene. The observation that all *trp–cysB* deletions inactivated *topA* indicated that *topA* either was within this interval or was very close to *trp* or *cysB*. Our findings that many, but not all, *cysB–pyrF* deletions inactivated *topA* demonstrated that this gene was not between *cysB* and *pyrF* but could be deleted without inactivation of the *trp* locus. The existence of deletions that simultaneously inactivated *topA* and either *trp* or *cysB* confirmed that *topA* was between and distinct from these closely linked loci.

The plasmids from the 5 Trp$^-$ isolates and from 13 of the Trp$^-$ Cys$^-$ derivatives of F′123 were transferred by conjugation into strains MT101 (*cysB12 pyrF146 endA41*::Tn*10*) or MT102 (*pyrF146 endA41*::Tn*10*). Of the 18 plasmids, only the one (pMT4410, Trp$^-$ topoisomerase$^+$) that had been capable of determining the synthesis of *E. coli* ω protein antigen in strain MT10 (*trpE52 cysB12 pyrF146*) now determined the synthesis of this protein in strains MT101 and MT102 (data not shown). Thus the ability or inability of the tested plasmids to specify production of this protein was not altered by the presence of wild-type *trp* or *trp–cysB* segments in the *S. typhimurium* chromosome.

Failure to Resolve _topA_ and _supX_. The only gene previously identified within the *trp–cysB* interval in either *E. coli* or *S.*

typhimurium is *supX*. Mutations in *supX* suppress the *S. typhimurium* promoter mutation *leu-500*, restoring the ability to grow in the absence of added leucine (20). The *E. coli supX* gene, present on F'123, can complement a mutant *supX* allele in the chromosome of a *S. typhimurium supX leu-500* strain, making this strain a leucine auxotroph (21).

The mutant episomes from the 5 Trp^- derivatives of TR5352 shown in Table 3 were transferred by conjugation into *S. typhimurium* strain PM214, which has a *supX–cysB* deletion and the *leu-500* allele. Cys^+ exconjugants were selected on plates that contained leucine and were then tested for a leucine requirement. Exconjugants that had received the episome from MT4410, which carries a wild-type *topA* gene, became leucine auxotrophs, indicating they had received a wild-type *supX* gene. Exconjugants that received episomes carrying *trp–topA* deletions from the other four donors did not become leucine auxotrophs, indicating that the episomal *supX* gene had been inactivated by each of these deletions. The failure of these deletions to resolve *topA* and *supX* is compatible with the possibility that the *E. coli topA* and *supX* genes are identical.

DISCUSSION

We have identified an *E. coli* gene, *topA*, that is required for production of *E. coli* DNA topoisomerase I antigen in *S. typhimurium*, and we have demonstrated that this gene is located between the *trp* operon and the *cysB* gene of the chromosomal segment carried by F'123. We contend that *topA* is the structural gene of *E. coli* DNA topoisomerase I and suggest that this gene may be identical to *supX*.

By strict immunological criteria, *S. typhimurium* strains that contain F'123 produce an antigen that is identical to *E. coli* DNA topoisomerase I and may produce more of this antigen than do haploid strains of *E. coli*. We reject the possibility that F'123 contains a gene that modifies *S. typhimurium* DNA topoisomerase I into a more competitive antigen on the grounds that it is impossible to convert a complex heterologous antigen into a more efficient competitor than the homologous antigen against which antiserum was raised. Instead, we conclude that F'123 carries the structural gene for *E. coli* DNA topoisomerase I and interpret the slight overproduction in *S. typhimurium* as a gene dosage effect. The *E. coli* chromosomal segment carried by this plasmid derives from a late replicating region adjacent to the terminus of chromosomal replication (22). Transfer of these genes to a single-copy plasmid could result in increased gene dosage through earlier replication of the episome. The observed *E. coli* ω protein content of TR5352 also indicates that a single gene can account for all of the DNA topoisomerase I found in haploid *E. coli* strains. Although we have not tested all regions of the *E. coli* genome for the ability to produce this protein, we presume that *topA* is the only structural gene for this enzyme. Unfortunately we have not yet succeeded in our attempts to delete the chromosomal *topA* gene by recombination with our deletion-bearing episomes, so we have not been able to test this presumption directly.

Our contention that *topA* is the structural gene that must be carried by F'123 is based on two main observations: *topA* is required for production of *E. coli* DNA topoisomerase I; and small deletions that eliminate *topA* are not complemented by the corresponding wild-type region of the *S. typhimurium* genome. If *topA* is not the structural gene, it must be a necessary accessory for expression of the structural gene located elsewhere on F'123. In this case, it must be able to activate the *E. coli* ω protein structural gene in concert with *S. typhimurium* enzymes and yet have no interchangeable counterpart in the *S. typhimurium* chromosome. In contrast, the structural gene hypothesis provides a simple and direct explanation for our data. Deletion of the structural gene prevents production of the *E. coli* ω protein. The exceptional strain MT5601, which appears to contain reduced amounts of the antigen, may have suffered a partial deletion of the structural gene and thus produces an antigenic fragment of this protein. Furthermore, we have preliminary data suggesting that plasmids other than F'123 that carry the *trp–cysB* region can produce *E. coli* ω protein.

Our suggestion that *topA* may be identical to *supX* rests less on our failure to resolve these genes, which is easily explained by other hypotheses, than on consideration of the pleiotropic phenotype of *supX* mutants. The demonstrated ability of *supX* to suppress mutations in various promoters (23) and to increase the levels of several unrelated enzymes (13, 24) is a plausible phenotype for mutations that inactivate DNA topoisomerase I. In the absence of the DNA-relaxing activity of this enzyme, DNA may be more underwound than usual, facilitating the binding of RNA polymerase to promoters (25) that otherwise would have a low affinity for the polymerase. If *E. coli topA* is identical with *E. coli supX*, then *S. typhimurium supX* is probably the structural gene for *S. typhimurium* DNA topoisomerase I. Therefore, selecting mutants with altered structural genes would be simple, and in the case of *S. typhimurium*, several nonsense mutations in *supX* have already been identified (13). This should allow us to learn how DNA topology is controlled and the consequences that ensue from disrupting this control.

The approach we have taken, using a radioimmunoassay and interspecies merodiploid strains, provides a powerful alternative to the use of enzyme activity assays or activity mutants, and should be readily applicable to locating the structural genes of other purified proteins that show sufficient interspecies antigenic variation. After completion of this work, we learned of experiments by R. Sternglanz *et al.* (26) with an *E. coli* mutant deficient in DNA topoisomerase I activity. Their results indicate that the mutation responsible for loss of enzyme activity is located near *trp*. The accordance of the present results and their findings, obtained by quite different methods, is reassuring.

Since this work was completed, it was shown in this laboratory that: (*i*) *S. typhimurium supX* mutations lead to the loss of the DNA topoisomerase I antigen; and (*ii*) multicopy hybrid plasmids pLC4-6, pLC5-23, pLC41-15 from the Clarke and Carbon colony bank (27), which carry the *trp–cysB* region of the *E. coli* chromosome, determine the synthesis of DNA topoisomerase I antigen.

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