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# *Escherichia coli* Strains Containing Mutations in the Structural Gene for Topoisomerase I Are Recombination Deficient

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**Mutations in the gene encoding topoisomerase I of *Escherichia coli* were tested for their effect on plasmid recombination. Recombination was decreased 1,000-fold at 30 and 37°C and occurred at approximately wild-type frequencies at 42°C. The suppression of *topA* mutations at 42°C did not appear to be a result of increased topoisomerase I activity at 42°C.**

*Escherichia coli* topoisomerase I (formerly ω-protein) is a 110,000-dalton protein capable of catalyzing the relaxation of negatively superhelical DNA (19), the knotting of single-stranded circular DNA (10), and the catenation of two duplex circular DNAs if one of the DNA molecules contains a single-strand scission (1, 18). A genetically and mechanistically distinct type of enzyme, topoisomerase II (formerly DNA gyrase) in *E. coli*, has also been identified in several organisms (6, 8). In addition to a superhelical relaxing activity (7), *E. coli* topoisomerase II has the diametrically opposed ability to catalyze the ATP-dependent introduction of negative supercoils into DNA (2). The associated activities of topoisomerases I and II suggest that the ability to regulate the superhelical density of DNA molecules is important to cellular function. Deletion mutants in the structural gene for topoisomerase I, designated *topA*, have been described (16) and have been shown to lead to an increase in the superhelical density of the DNA present in the mutants (12). The interdependence of *E. coli* topoisomerases was demonstrated when *E. coli* strains containing deletion mutants covering the gene for topoisomerase I were found to contain compensatory mutations in *gyrA* or *gyrB*, the structural genes for topoisomerase II (4, 12). The compensatory mutations in the *gyrA* or *gyrB* loci apparently reduce, rather than eliminate, the activity of topoisomerase II, since it is known that mutations which eliminate topoisomerase II activity are conditionally lethal (11). Besides being critical for DNA replication and site-specific recombination, the regulation of superhelix density also appears to be critical for transcription (15). This was first demonstrated when the *supX* mutation was isolated in *Salmonella typhimurium* as a suppressor of promoter mutations in the leucine biosynthetic pathway and was later mapped to the *topA* locus (17). Presumably, the phenotype of *supX* is a result of increased negative superhelical density that facilitates RNA polymerase binding and subsequent transcription of the DNA. It also appears that many other types of second-site mutations can occur in *topA E. coli*. Repeated passage of topoisomerase I-deficient *E. coli* on rich media eventually results in a strain that can no longer survive on a minimal medium in which the original parent strain could grow (unpublished data). This observation suggests that the presence of *topA* mutations results in a selection for altered expression of metabolic genes.

Topoisomerase I has also been found to participate in reactions that are catalyzed by other DNA enzymes in vitro and halted at some intermediate stage as a result of topological inhibition. An example is the topological linking of circular duplex and circular single-stranded DNA, annealed by homologous pairing and catalyzed by the *E. coli recA* protein (13). This reaction may be related to the mechanisms of initiation of postreplication repair and general genetic recombination in vivo. To test the effect of topoisomerase I mutations on plasmid recombination, the isogenic set of strains listed in Table 1 were examined for their recombination proficiency. Strain SY791 was used in our experiments because it lacked a second-site mutation that we found to be present in parent strain DM700 and in other topoisomerase I-deficient strains examined, which precluded their use. We have mapped this mutation (unpublished data) to the *acrA* gene locus, which is known to confer hypersensitivity to acridines and tetracycline.

Table 2 shows the frequency of plasmid recombination determined for the set of isogenic strains described in Table 1, using pRDK301 as a substrate(s). Plasmid pRDK301 is a tandemly repeated dimer of pBR322 that contains two different mutant tetracycline resistance genes. Recombination of these allelic mutations to produce a wild-type, tetracycline-resistant gene product was used to determine the frequency of plasmid recombination as previously described

TABLE 1. Sources of bacteria and plasmids

| Bacterium or plasmid | Genotype | Source |
|---|---|---|
| Bacteria | | |
| W3110 | *thyA* | Laboratory stock |
| DM700 | W3110 Δ(*top-cys*)[a] | J. C. Wang |
| XA90 | Δ(*lac-pro*)*X111* F⁻ *argE(Am) araD nalA* | J. Beckwith |
| SY791 | XA90 Δ(*top-cys*)*700* | R. Isberg, M. Syvanen |
| SY792 | XA90 *top-10* | R. Isberg, M. Syvanen |
| SY797 | XA90 *trp*::Tn*10* | R. Isberg, M. Syvanen |
| Plasmids | | |
| pACYC184 | Cm$^r$ Tc$^r$ | A. C. Y. Chang and S. N. Cohen |
| pRDK301 | Ap$^r$ Tc$^s$ | R. Fishel et al. |
| pJW249 | CysB$^+$ Top$^+$ | J. C. Wang (20) |

[a] Contains known second-site mutations.

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TABLE 2. Plasmid recombination in topoisomerase I-deficient *E. coli*

| Strain | Recombination frequency[a] | Mutant/ wild type |
|---|---|---|
| XAc (wild type) | $4.4 \times 10^{-3}$ | 1.00 |
| SY797 *top-10* | $2.54 \times 10^{-3}$ | 0.58 |
| SY791 Δ(*top-cys*) | $4.37 \times 10^{-6}$ | 0.00099 |
| SY791(pJW249)[b] | $4.06 \times 10^{-3}$ | 0.923 |

[a] *E. coli* strains of interest were transformed with pRDK301, and transformants were selected on L broth plates supplemented with 50 μg of ampicillin per ml at 37°C. Several individual colonies were dissected from the selection plate and allowed to grow separately to an optical density at 590 nm of 0.6 at 37°C (ca. 30 generations total). The frequency of plasmid recombination was determined by measuring the frequency of $Ap^r$ $Tc^r$ cells by the method of Fishel et al. (5) at 37°C. The average of several independent determinations are described.

[b] Defective F-factor derivative containing chromosomal insert with the structural gene for topoisomerase I and the *cysB* gene locus. This low-copy plasmid is compatible with pBR322.

(5). A 1,000-fold reduction in the frequency of plasmid recombination was found in *E. coli* SY791, which contains a deletion of the topoisomerase I locus. The reduction in plasmid recombination could be fully complemented if the low-copy plasmid pJW249 (20), containing the cloned topoisomerase I gene, was introduced into SY791. This result suggests that the superhelical configuration of plasmid parents participating in genetic recombination is important for the generation of recombinants. Alternately, a deficiency in topoisomerase I could affect the expression of proteins required for plasmid recombination. SY797 was found to reduce plasmid recombination only slightly. This observation is consistent with the high level of residual topoisomerase I activity present in cell extracts of this strain (see Fig. 2).

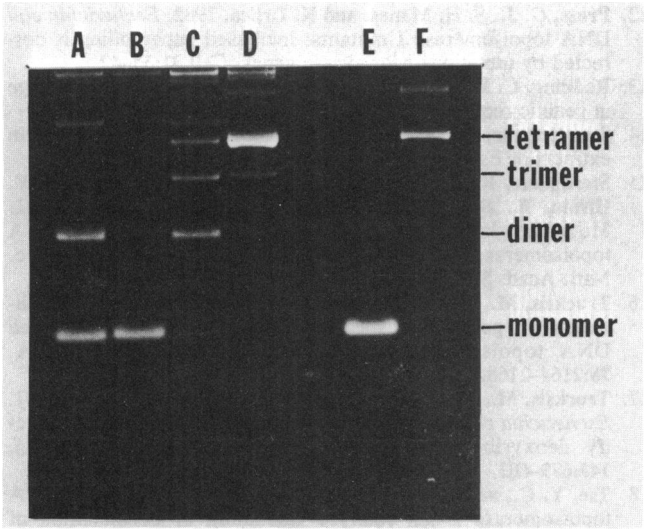


FIG. 1. Oligomeric interconversion in topoisomerase I-deficient *E. coli*. The extent of oligomeric interconversion was measured by the method of James et al. (9), using purified monomeric or tetrameric pACYC184. Several individual transformants of each strain were examined. Representative DNA preparations are shown in lanes A through D. Lanes: A, monomer pACYC184 introduced into XA90 wild type; B, monomer pACYC184 introduced into SY791 Δ(*top-cys*)*700*; C, tetrameric pACYC184 introduced into XA90 wild type; D, tetrameric pACYC184 introduced into SY791 Δ(*top-cys*)*700*.

A second method for the measurement of genetic recombination is the examination of oligomeric interconversion (9). Figure 1 shows the extent of oligomeric interconversion that occurred in strain SY791 when either purified monomer (lane B) or tetramer (lane C) pACYC184 DNA was introduced. The results showed that both the conversion of monomeric pACYC184 to higher oligomeric forms and the conversion of tetrameric pACYC184 to lower oligomeric forms was reduced in topoisomerase I deletion strain SY791. The deficiency in the interconversion of circular oligomers observed with the SY791 *topA* strain could be comple-

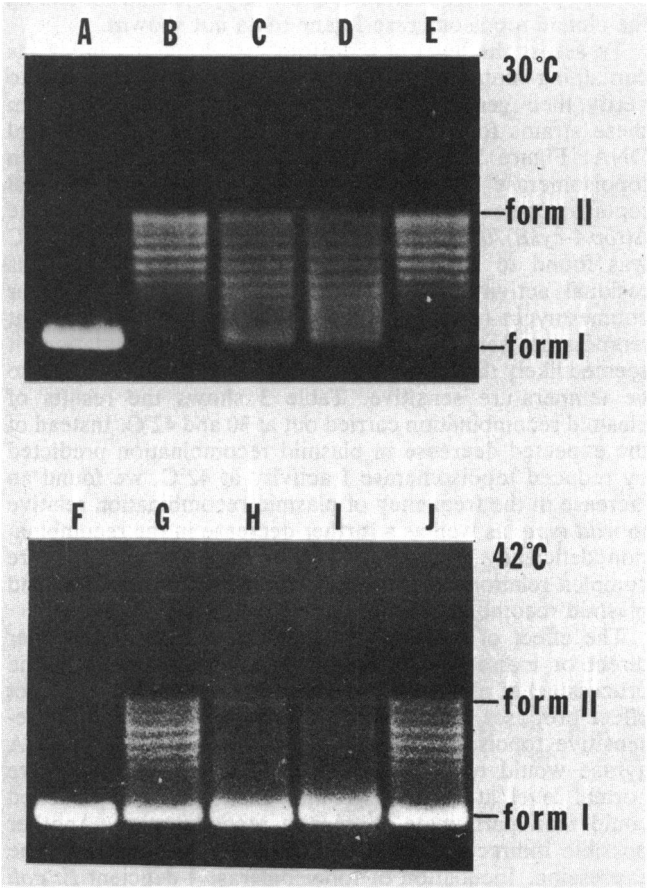


FIG. 2. Assay of topoisomerase I activity in several *E. coli* strains. Cell extracts were prepared by a modification of the method of Staudenbaur (14). *E. coli* cells (50 ml; absorbance at 590 nm, 1.0) were harvested, suspended in 1/100 volume of 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 8.0)–5 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*-tetraacetic acid (EGTA), and frozen in liquid nitrogen. The thawed cell suspension was made 0.15 M NaCl, 0.1% phenylmethylsulfonyl fluoride, 10 mM β-2-mercaptoethanol, and 200 μg of lysozyme per ml; incubated on ice for 45 min; frozen in liquid nitrogen; and thawed and centrifuged at 150,000 × *g* for 10 min at 4°C. Glycerol (in 25 mM HEPES [pH 8.0]–5 mM EGTA) was added to a final concentration of 10% (wt/vol). Topoisomerase I activity was assayed in 8.5 mM Tris (pH 8.0)–50 mM KCl–5 mM $MgCl_2$–50 μg of *E. coli* B tRNA–100 μg of bovine serum albumin per ml–10 μg of supercoiled pBR322 per ml. 200-μg portion of the total protein of each extract described was added, and incubation was performed at the temperature shown for 60 min. Topoisomerases produced were then separated by electrophoresis in 1.0% agarose slab gels, followed by staining in 0.5 μg of ethidium bromide per ml. Lanes: A and F, unincubated controls; B and G, wild type XA90; C and H, SY792 *top-10*; D and I, SY791 *top-700*; E and J, SY791 *top-700* plus p2VS49.

TABLE 3. Effect of temperature on plasmid recombination[a]

| Strain | Frequency of recombination at 30°C | Mutant/wild type ratio at 30°C | Frequency of recombination at 42°C | Mutant/wild type ratio at 42°C |
|---|---|---|---|---|
| χA90 (wild type) | $7.3 \times 10^{-4}$ | 1.00 | $6.1 \times 10^{-3}$ | 1.00 |
| SY791 [Δ(*top-cys*)*700*] | $2.9 \times 10^{-7}$ | 0.0004 | $2.2 \times 10^{-3}$ | 0.37 |

[a] Frequencies were measured by the method of Fishel et al. (5) and as described in footnote *a* of Table 2.

mented by the introduction of a low-copy plasmid containing the cloned topoisomerase I gene (data not shown).

To assess the level of topoisomerase I activity in strains containing mutations in the topoisomerase I gene and to verify their genotype, we assayed lysates prepared from these strains for relaxing activity on superhelical plasmid DNA. Figure 2 illustrates the effect of temperature on topoisomerase I activity in vitro. The significant residual topoisomerase I activity observed in strains containing the Δ(*topA-cysB*)*700* deletion or the *top10* point mutation at 30°C was found to be decreased or eliminated at 42°C. This residual activity was not sensitive to naladixic acid or coumermycin (data not shown). Because of the apparent temperature sensitivity of the topoisomerase I mutations it seemed likely that plasmid genetic recombination would also be temperature sensitive. Table 3 shows the results of plasmid recombination carried out at 30 and 42°C. Instead of the expected decrease in plasmid recombination predicted by reduced topoisomerase I activity at 42°C, we found an increase in the frequency of plasmid recombination relative to *wild type*, as well as a further decrease in the recombination deficiency at 30°C. These results suggested a more complex relationship between topoisomerase I activity and plasmid recombination.

The effect of temperature could be the result of either direct or indirect participation of topoisomerase I in the process(es) of plasmid recombination. One possible indirect effect proposes that strain SY791 contains a temperature-sensitive topoisomerase II. A temperature-sensitive DNA gyrase would reduce the superhelical density to a more normal level at 42°C. DNA that is normally supercoiled could then participate in genetic recombination. Another possible indirect effect would be a result of altered gene expression. Incubation of topoisomerase I-deficient *E. coli* at high temperature would presumably alleviate reduced gene expression in a manner similar to *supX* or by allowing transcription through increased promoter "melting." Finally, topoisomerase I could participate directly in plasmid genetic recombination. The absence of topoisomerase I at high temperature would then be complemented by the activation or inactivation of another protein. A possible candidate for an activated second protein is topoisomerase III, which has been shown to be more active at higher temperatures (3). An exact determination of the role of topoisomerase I in plasmid recombination will most likely await its study in an in vitro recombination system to eliminate the possibility of indirect effects.

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## LITERATURE CITED

1. **Brown, P. O., and N. R. Cozzarelli.** 1981. Catenation and knotting of duplex DNA by type I topoisomerases. Proc. Natl. Acad. Sci. U.S.A. **78**:843–847.
2. **Cozzarelli, N. R.** 1980. DNA gyrase and the supercoiling of DNA. Science **207**:953–960.
3. **Dean, F., M. A. Krasnow, R. Otter, M. M. Matzuk, S. J. Spengler, and N. R. Cozzarelli.** 1983. *Escherichia coli* type-I topoisomerases: identification, mechanism, and role in recombination. Cold Spring Harbor Symp. Quant. Biol. **47**:769–777.
4. **DiNardo, S., K. A. Voelkel, and R. Sternglanz.** 1982. *Escherichia coli* DNA topoisomerase I mutants have compensatory mutations in DNA gyrase genes. Cell **31**:43–51.
5. **Fishel, R. A., A. A. James, and R. Kolodner.** 1981. *Rec*A-independent general genetic recombination of plasmids. Nature (London) **294**:184–186.
6. **Gellert, M.** 1981. DNA Topoisomerases. Ann. Rev. Biochem. (Snell, E. E., P. D. Boyer, A. Meister, and C. C. Richardson, eds.). **50**:879–910.
7. **Gellert, M., L. M. Fisher, and M. H. O'Dea.** 1979. DNA Gyrase: Purification and catalytic properties of a fragment of gyrase B protein. **76**:6289–6293.
8. **Gellert, M., K. Mizuuchi, M. H. O'Dea, and H. A. Nash.** 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. U.S.A. **73**:3872–3876.
9. **James, A. A., P.T. Morrison, and R. Kolodner.** 1982. Genetic recombination of bacterial plasmid DNA: analysis of the effect of recombination-deficient mutations on plasmid recombination. J. Mol. Biol. **160**:411–430.
10. **Liu, L. F., R. E. Depew, and J. C. Wang.** 1976. Knotted single-stranded DNA rings: a novel topological isomer of circular single-stranded DNA formed by treatment with *E. coli* protein. J. Mol. Biol. **106**:439–452.
11. **Menzel, R., and M. Gellert.** 1983. Regulation of the genes for *E. coli* DNA gyrase: homeostatic control of DNA supercoiling. Cell **34**:105–113.
12. **Pruss, G. J., S. H. Manes, and K. Drlica.** 1982. *Escherichia coli* DNA topoisomerase I mutants: increased supercoiling is corrected by mutations near gyrase genes. Cell **31**:35–42.
13. **Radding, C. M.** 1982. Homologous pairing and strand exchange in genetic recombination. Annu. Rev. Genet. **16**:405–437.
14. **Staudenbaur, W. L.** 1976. Replication of small plasmids in extracts of *Escherichia coli*. Mol. Gen. Genet. **145**:273–280.
15. **Sternglanz, R., S. DiNardo, K. A. Voelkel, Y. Nishimura, Y. Hirota, K. Becherer, L. Zumstein, and J. C. Wang.** 1981. Mutations in the gene coding of *Escherichia coli* DNA topoisomerase I affect transcription and transposition. Proc. Natl. Acad. Sci. U.S.A. **78**:2747–2751.
16. **Trucksis, M., and R. E. Depew.** 1981. Identification and localization of a gene which specifies production of *Escherichia coli* DNA topoisomerase I. Proc. Natl. Acad. Sci. U.S.A. **78**:2164–2168.
17. **Trucksis, M., E. I. Golub, D. J. Zabel, and R. E. Depew.** 1981. *Escherichia coli* and *Salmonella typhimurium Sup*X genes specify deoxyribonucleic acid topoisomerase I. J. Bacteriol. **147**:679–681.
18. **Tse, Y.-C., and J. C. Wang.** 1980. *E. coli* and *M. Luteus* DNA topoisomerase I can catalyze catenation or decatenation of double-stranded DNA rings. Cell **22**:269–276.
19. **Wang, J. C.** 1971. Interaction between DNA and *Escherichia coli* protein. J. Mol. Biol. **55**:523–533.
20. **Wang, J. C., and K. Becherer.** 1983. Cloning of the gene *top*A encoding DNA topoisomerase I and physical mapping of the *cys*B-*top*A-*trp* region of *Escherichia coli*. Nucleic Acids Res. **11**:1773–1790.