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Expression of yeast DNA topoisomerase I can complement a conditional-lethal DNA topoisomerase I mutation in *Escherichia coli*

(DNA topology/DNA supercoiling/antitumor drugs)

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ABSTRACT **We show that, despite differences in primary structure, substrate preference, and mechanism of catalysis, yeast DNA topoisomerase I can functionally substitute for *Escherichia coli* DNA topoisomerase I. A family of plasmids expressing the yeast *TOP1* gene or 5′-deletion mutations of it were used to complement the temperature-sensitive phenotype of an *E. coli topA* mutant. These plasmids were then isolated from the cells by a rapid lysis procedure and examined for their degrees of supercoiling. Functional complementation of a conditional-lethal mutation in *topA*, which encodes *E. coli* DNA topoisomerase I, correlates with the expression of a catalytically active yeast enzyme that reduces the degree of negative supercoiling of intracellular DNA. We also show that ≈130 amino acids of the amino-terminal portion of the yeast enzyme can be deleted without affecting its activity *in vitro*; activity of the enzyme inside *E. coli*, however, is more sensitive to such deletions.**

In eubacteria, as well as in eukaryotes, two classes of DNA topoisomerases have been well-characterized (for reviews, see refs. 1–4). The type I topoisomerases break and rejoin one DNA strand at a time in the interconversion of the topological isomers (topoisomers) of DNA rings or loops. In eubacteria, the major type I activity is DNA topoisomerase I; a second type I topoisomerase, DNA topoisomerase III, has been found in *Escherichia coli* (5, 6), but a detailed characterization of this enzyme is lacking. In eukaryotes, only one type I enzyme, DNA topoisomerase I, is known.

Bacterial and eukaryotic DNA topoisomerase I differ in several respects and appear to be unrelated structurally. The eukaryotic enzyme interacts preferentially with double-stranded DNA (7) and relaxes positively and negatively supercoiled DNAs to completion (8, 9). When the enzyme transiently cleaves DNA, it forms a covalent intermediate in which the enzyme is linked to a 3′-phosphoryl end of the cleaved DNA strand (10). The prokaryotic enzyme, on the other hand, normally relaxes negatively supercoiled DNA (11); positively supercoiled DNA is relaxed only if a single-stranded loop is present (12). The bacterial enzyme becomes covalently linked to a 5′-phosphoryl group when it transiently cleaves DNA (13). The differences in substrate preference and catalysis between the eukaryotic and prokaryotic enzymes are consistent with the apparent lack of similarity between the amino acid sequences of *E. coli* topoisomerase I and the yeast *Saccharomyces cerevisiae* topoisomerase I (14, 15).

The type II topoisomerases are exemplified by bacterial gyrase and eukaryotic topoisomerase II. They are classified as type II enzymes by virtue of their concerted breakage and rejoining of both strands of a duplex DNA (16, 17). Whereas both enzymes require ATP, only bacterial gyrase (an A_2B_2 tetramer) can utilize the chemical energy of ATP hydrolysis for the negative supercoiling of DNA—i.e., the reduction of the linking number of a double-stranded DNA ring to values below that of the same DNA in its most stable configuration. The eukaryotic enzyme (a homodimer) can relax positively or negatively supercoiled DNA but cannot supercoil a relaxed DNA. In contrast to the case of the eubacterial and eukaryotic type I enzymes, gene sequences reveal evolutionary and structural similarities between the type II enzymes (reviewed in ref. 4).

Functionally, bacterial DNA gyrase is essential (reviewed in refs. 16–18). In *E. coli*, DNA topoisomerase I also affects cell viability. *E. coli topA* deletion mutants are viable only in the presence of a second compensatory mutation, which often reduces the supercoiling activity of gyrase (19–21). The compensatory mutation requirement for the survival of *topA* mutants can be substituted by a sublethal dose of a gyrase inhibitor (A. F. Stankiewicz and R. E. Depew, personal communication). These and other results support the notion that the regulation of the degree of supercoiling of intracellular DNA in *E. coli* is essential for cell viability.

In this communication, we show that despite many differences between the eukaryotic and prokaryotic type I enzymes, the expression of the yeast *TOP1* gene in *E. coli* can complement a conditional-lethal *E. coli topA* mutant, AS17.* Examination of the degrees of supercoiling of plasmid DNAs isolated from *E. coli* cells expressing the yeast gene indicates that relaxation of intracellular DNA by the yeast enzyme is essential for functional complementation. We also show that ≈130 amino acids of the amino-terminal portion of the yeast enzyme can be deleted without significantly affecting its activity *in vitro*. The ability of the yeast enzyme to relax intracellular DNA in *E. coli* is, however, more sensitive to such deletions.

MATERIALS AND METHODS

Materials, Bacterial Strains, and Construction of Yeast TOP1 Clones. *E. coli* strains MM294 (*thi endA* hsr_k) and NK7048 [($\Delta lac\ pro$)$_{XIII}$ *ara nalA* $argE_{am}$ *thi* rif^R F′ $lacI^{q1}$)] were from M. Meselson and N. Kleckner (Harvard University), respectively. *E. coli* strain AS17 [$topA_{am}$PLL1-($Tc^R supD^{ts}$)] was kindly provided by R. E. Depew (Northeastern Ohio Universities). AS17 exhibits a temperature-sensitive-lethal phenotype that is rescued by $topA^+$ plasmids. In this study, the permissive and nonpermissive temperatures refer to 30°C and 37°C, respectively; the nonpermissive temperature normally employed for AS17, 42°C, is partially inhibitory to yeast topoisomerase I activity.

Expression of yeast DNA topoisomerase I in *E. coli* was accomplished by placing the yeast *TOP1* gene under the

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Abbreviation: IPTG, isopropyl β-D-thiogalactoside.

*Stankiewicz, A. F. & Depew, R. E., 83rd Annual Meeting of the American Society of Microbiology, March 6–11, 1983, New Orleans, p. 122, abstr. H102.

control of the *E. coli tac* promoter, which is regulated by the *lac* repressor. The starting material, plasmid pTP1, was derived from the *TOP1* plasmid pIG-1 (22) by digesting the plasmid with *Pvu* II and *Sna*BI and linking the ends through an octameric *Xho* I linker (New England Biolabs). pTP1 was then digested with *Sfa*NI and repaired with *E. coli* DNA polymerase I. The 1150-base pair restriction endonuclease fragment starting with the second triplet of the coding sequence of the yeast enzyme was isolated by electrophoresis in a 4% polyacrylamide gel. The expression plasmid ptac12B (23), a derivative of pBR322 containing the *trp-lac* fusion promoter *tac*, a ribosome binding site, and an *Nco* I site downstream of it, was digested with *Nco* I and then repaired with *E. coli* DNA polymerase I. The *Sfa*NI fragment was then inserted into the expression vector to join the second coding triplet to the initiation codon ATG at the end of the repaired *Nco* I site. The resulting plasmid was isolated from a *dam* strain and digested with *Bcl* I and *Pvu* I. The smaller fragment from the digestion was then ligated to the larger *Bcl* I to *Pvu* I fragment obtained from pTP1, which was also isolated from a *dam* strain. The resulting plasmid ptacTOP1 contains the *tac* promoter and its downstream ribosome binding site, the yeast *TOP1* sequence from the first ATG to the *Sna*BI site past the coding sequence of the yeast enzyme, and 2300 base pairs of pBR322 DNA from the *Pvu* II site to the *Eco*RI site (see Fig. 1).

To construct 5′ deletions in the *TOP1* gene, plasmid pTP1-4, which contains a *Bam*HI linker inserted into the *Sst* I site at position −22 in the yeast *TOP1* sequence (numbered from the first ATG of the coding sequence), was linearized with *Bam*HI. The DNA was treated with BAL-31 nuclease (New England Biolabs), and the reaction was terminated at various times by phenol extraction and ethanol precipitation. The DNA ends, which were filled in with the Klenow fragment of DNA polymerase I, were subsequently ligated together in the presence of an excess of phosphorylated *Nco* I linkers. These recircularized DNAs were transformed into *E. coli* strain MM294. Each mixture of transformants was grown in Luria broth plus ampicillin (or carbenicillin), and plasmid DNA was obtained by the NaOH/$NaDodSO_4$ lysis procedure (24). The rationale for this step was to increase the DNA concentrations for the subsequent low-efficiency transformations of strain AS17. The *Nco* I–*Pvu* I fragments of the MM294 "amplified" DNAs, containing the *TOP1* deletions, were gel purified in low-melting-temperature agarose and ligated with the gel-purified *Pvu* I–*Nco* I fragment of plasmid ptac12B, thus placing the yeast topoisomerase I deletions downstream from the *tac* promoter. Following transformations of strain AS17 with these DNAs, ampicillin-resistant colonies were selected and assayed individually for the presence of yeast topoisomerase I antigenic determinants as described below. Total cellular proteins were resolved by $NaDodSO_4$/polyacrylamide gel electrophoresis (25), electroblotted onto nitrocellulose sheets, and immunostained using rabbit antibodies against yeast DNA topoisomerase I and horseradish peroxidase-based immunodetection reagents (Bio-Rad).

Topoisomerase I Activity Assays. Bacterial cell extracts for activity assays were prepared from 10 ml of overnight cultures as described (15, 26).

E. coli topoisomerase I activity in 2 μl of a 1:10 dilution of the extract was assayed in a total volume of 20 μl containing 0.2 μg of a 2-kilobase-pair negatively supercoiled plasmid pHC624 DNA, 28 mM Tris·HCl (pH 8.0), 70 mM NaCl, 3.5 mM $MgCl_2$, 0.3 mM Na_2EDTA, gelatin (35 μg/ml), and tRNA (70 μg/ml), at 37°C for 45 min (modified from ref. 27). Yeast topoisomerase I relaxation activity was assayed with 0.2 μg of the same DNA in 20 μl of 20 mM Tri·HCl (pH 7.5), 10 mM Na_2EDTA, 150 mM KCl, 1 mM 2-mercaptoethanol, and gelatin (50 μg/ml) at 30°C for 60 min (28). The omission of Mg^{2+} in the assay mixture ensures that the DNA relaxation activity assayed is that of the yeast enzyme, since the divalent metal ion is required by all known *E. coli* DNA topoisomerases (1, 2). In both instances, the reaction was terminated by the addition of $NaDodSO_4$ to 1% and heating at 60°C for 2 min. The extent of DNA relaxation was assayed by electrophoresis in a 0.7% agarose gel as described (29).

***In Vivo* Complementation.** Complementation of the AS17 temperature-sensitive growth phenotype by plasmids expressing the yeast *TOP1* gene or its deletion mutations was determined by assaying cell viability at 30°C and 37°C. AS17 transformants were grown in Luria broth containing ampicillin or carbenicillin. Serial dilutions of these cultures were plated and incubated at the permissive and nonpermissive temperatures, and the number of cells forming colonies were counted.

Rapid Lysis of Cells and the Isolation of Plasmids for Linking-Number Measurements. The NaOH/$NaDodSO_4$ lysis procedure (24) was adapted for the rapid lysis of cells. Basically, 100 ml of cultures in 500-ml Erlenmeyer flasks on a gyratory shaker (New Brunswick) were rapidly lysed by pouring in an equal volume of a mixture containing 2–4% (wt/vol) $NaDodSO_4$ and 0.2 M NaOH. Following neutralization and precipitation of the detergent with 75 ml of 5 M potassium acetate (pH 4.8), the supernatant was cleared by centrifugation at 5000 × *g* for 10 min and filtered through cheesecloth. The DNA was precipitated with 2 vol of ethanol and pelleted at 12,000 × *g* for 15 min. Plasmid DNA was subsequently purified by banding in CsCl/ethidium gradients (30), at 150,000 × *g*; a broad layer below the nicked DNA band was collected to minimize the preferential loss of covalently closed topoisomers with linking numbers that differ significantly from the predominant topoisomers. After the removal of CsCl and ethidium, the linking number distributions of the DNA samples were analyzed by two-dimensional gel electrophoresis as described (31), except chloroquine was present during the first- as well as the second-dimension electrophoresis. Electrophoresis conditions and chloroquine concentrations are given in the figures.

RESULTS

Plasmids Expressing Yeast DNA Topoisomerase I in *E. coli* Were Used To Show That a Portion of the Amino Terminus of the Enzyme Can Be Deleted Without Inactivating It. Fig. 1 depicts the general construct of the ptacTOP1 plasmids used in this work. In each case, all or a portion of the coding sequence of the yeast gene is placed downstream of a *lac* repressor-regulated *tac* promoter in an expression vector ptac12B (23); an ATG triplet, originally part of an *Nco* I restriction site downstream of the *tac* promoter, serves as the initiation codon.

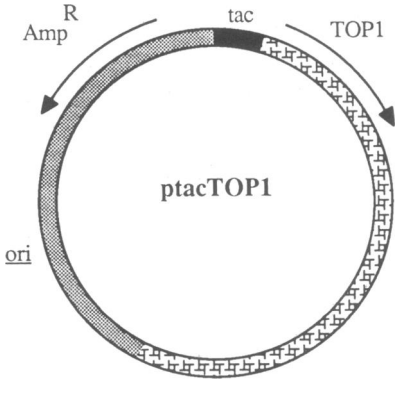

FIG. 1. The general construct of ptacTOP1 plasmids. Amp^R, ampicillin resistance; *ori*, origin.

In a *lacI*$^+$ strain such as MM294 or AS17, the multicopy *tac* plasmids express a high level of message even in the absence of isopropyl β-D-thiogalactoside (IPTG) induction. In strain NK7048, which contains a *lacI*^{q1} gene on an F′ episome, transcription from the *tac* promoter is repressed; efficient expression of the yeast enzyme requires IPTG. It appears that the overexpression of yeast topoisomerase I in *E. coli* is deleterious, as is the overexpression of *E. coli* DNA topoisomerase I (30). Thus, unless stated otherwise, IPTG is absent during the growth of cells containing ptacTOP1.

Table 1 lists the results of activity assays in extracts of AS17 cells harboring various plasmids. For convenience, each of the 5′ deletions of ptacTOP1 is designated by ptacTOP1Δ*n*, where *n* is the approximate number of base pairs deleted from the 5′ end of the *TOP1* gene. Clearly, deletion of up to 415 base pairs or 18% of the *TOP1* gene from the 5′ terminus of the coding sequence does not inactivate the yeast enzyme.

Yeast DNA Topoisomerase I Can Complement a Conditional-Lethal *E. coli* topA Mutation. *E. coli* strain AS17 *top*-A_{am}pLL1(*supD*$_{ts}$) exhibits a conditionally lethal *topA* phenotype, resulting from the temperature-sensitive supression of a *topA* amber mutation.* As shown in Table 1, increasing the incubation temperature from 30°C to 37°C decreases the viability of AS17 cells by >100-fold. This temperature-sensitive phenotype is rescued, however, by transforming the strain with ptacTOP1 or ptacTOP1a, both of which express the entire coding sequence of yeast DNA topoisomerase I. Some of the ptacTOP1 derivatives with portions of the 5′-terminal coding sequences deleted can also complement the *topA ts* phenotype.

Results tabulated in Table 1 suggest that the genetic complementation of *topA* by *TOP1* requires the production of enzymatically active yeast DNA topoisomerase I. The frameshift mutant ptacTOP1Δ75fs, for example, is completely ineffective in rescuing the *topA ts* phenotype. All of the plasmids that can restore AS17 cell viability yield active yeast DNA topoisomerase I in extracts. However, the converse is not true: plasmids that express amino-terminal deletions of the yeast enzyme active in cell extracts are not always effective in complementation; examples are plasmids ptacTOP1Δ385a, Δ400, and Δ415.

Table 1. Activity of yeast DNA topoisomerase I in extracts of *E. coli* strain AS17 *topA*$_{am}$ pLL1(*supD*^{ts}) cells harboring various plamids and the effect of these plasmids on AS17 cell viability

Plasmid	Relaxation activity in cell extracts	Cell viability
ptacTOP1	+++	1.7
ptacTOP1a	+++	3.8
ptacTOP1Δ50	nd	3.7
ptacTOP1Δ75	+++	0.6
ptacTOP1Δ200a	++	1.0
ptacTOP1Δ200b	++	nd
ptacTOP1Δ385a	+++	<0.002*
ptacTOP1Δ385b	++	nd
ptacTOP1Δ400	++	<0.002*
ptacTOP1Δ415	+	<0.002*
ptacTOP1Δ75fs†	−	<0.002
None	−	0.005
pBR322Δ*Cla*‡	−	0.007

Serial dilutions of cell extracts were assayed. nd, Not determined. Cell viability refers to the ratio of the number of colonies at 37°C to the number of colonies at 30°C. −, undetectable activity; +, ++, and +++, increasing levels of activity.

*Prolonged incubation gave small colonies of ptacTOP1Δ385a and Δ400 transformants, increasing the ratio to 0.05; no increment of colonies was observed for ptacTOP1Δ415 transformants.

†A frameshift mutation was introduced in the deletion construction: no yeast DNA topoisomerase I antigenic determinants were detected in immunoblots of cell extracts.

‡Derived from pBR322 by the deletion of 2 base pairs within the *Cla* I site.

Complementation of the Conditional Lethal topA Phenotype Requires the Expression of Active Yeast DNA Topoisomerase I *in Vivo*. To test the possibility that the failure of Δ385a, Δ400, and Δ415 to complement AS17 may be due to the expression of the yeast protein in a form active *in vitro* but not *in vivo*, we examined the degrees of supercoiling of these plasmids recovered from *E. coli* NK7048 *topA*$^+$ *lacI*^{q1} cells.

A rapid lysis procedure was used to minimize the possibility of altering the linking numbers of the plasmids during or after cell lysis. Fig. 2 shows the electrophoresis patterns of several samples in an agarose gel. The electrophoresis buffer contained an optimal concentration of chloroquine for the resolution of topoisomers of different linking numbers. Lanes 1 and 2 are ptacTOP1 samples recovered from exponentially growing NK7048 cells in the absence and presence of IPTG, respectively. Because of the *lacI*^{q1} superrepressor mutation in the host strain, *TOP1* expression, though detectable, is at a low level in the absence of IPTG (lane 1). Induction of the plasmid-borne yeast *TOP1* gene by IPTG results in a general increase in intensity of topoisomer bands toward the nicked DNA band at the top of the ladder (lane 2), indicating a reduction in the degree of negative supercoiling

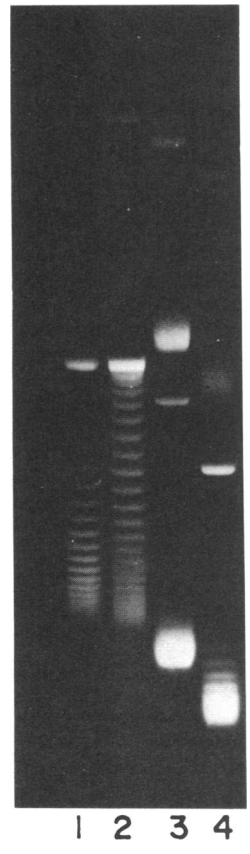

FIG. 2. Yeast DNA topoisomerase I-catalyzed relaxation of plasmid DNA inside *E. coli*. Plasmid DNA was purified from exponentially growing *E. coli* NK7048 cells, induced for 1–2 hr with 1 mM IPTG when indicated, according to the rapid-lysis method. Samples of DNA were electrophoresed for 20 hr at 2.5 V/cm in a 0.7% agarose gel in 0.1 M Tris base/0.1 M sodium borate/2 mM Na_2EDTA/1.9 μM chloroquine, pH 8.3. Following several washes in deionized water to remove the chloroquine, the gel was stained with ethidium bromide (1 μg/ml) and photographed over a UV source. Lanes 1 and 2 contain plasmid ptacTOP1 DNA from uninduced and IPTG-induced cells, respectively. Lanes 3 and 4 contain ptacTOP1Δ415 DNA and pBR322ΔC1a DNA, respectively, from IPTG-induced cells.

of the intracellular plasmid. Lanes 3 and 4 contain ptacTOP1Δ415 DNA and pBR322Δ*Cla* DNA, respectively, from IPTG-induced cells. Both are more negatively supercoiled relative to the ptacTOP1 DNA recovered from IPTG-induced cells. Thus, ptacTOP1Δ415 apparently expresses a truncated yeast DNA topoisomerase inactive in *E. coli* but active in cell lysates.

Positively and negatively supercoiled topoisomers with linking differences of the same absolute values are often unresolved by one-dimensional agarose gel electrophoresis, which introduces some uncertainty into the interpretation of the results depicted in Fig. 2. Two-dimensional agarose gel electrophoresis of the same samples, however, resolves all topoisomers (31, 32). On the left of Fig. 3, the arc of spots are topoisomers of ptacTOP1 purified from uninduced NK7048 *topA*$^+$ *lacI*^{q1} cells. The spot marked by an arrow is nicked ptacTOP1 DNA. The lower tip of the arc corresponds to topoisomers of the lowest linking numbers (i.e., the most negatively supercoiled). The linking numbers increase as the spots move up along the arc, and adjacent spots differ by one in their linking numbers (see refs. 1 and 31 for further interpretation of the two-dimensional patterns). Upon induction of *TOP1*, the arc of spots extends toward higher linking numbers (in the center of Fig. 3). The high linking-number end of the arc corresponds closely to topoisomers of ptacTOP1 completely relaxed with extracts of *E. coli* cells expressing yeast topoisomerase I in 20 mM Tris·HCl, pH 7.5/125 mM KCl/10 mM $MgCl_2$ (results not shown). In contrast to the broad arc of spots shown in the center of Fig. 3 for ptacTOP1 recovered from cells expressing *TOP1*, intracellular ptacTOP1Δ415 DNA remains highly negatively supercoiled following IPTG induction (on the right of Fig. 3). The two-dimensional results shown in Fig. 3 confirm the interpretation of the one-dimensional results (Fig. 2) discussed earlier. In a similar experiment, the distribution of ptacTOP1Δ385a topoisomers were also shown to be little affected by IPTG induction, demonstrating that ptacTOP1Δ385a also expresses yeast DNA topoisomerase I in *E. coli* in a form that is inactive *in vivo* but active *in vitro*.

In the analysis of plasmid topoisomer distributions *in vivo*, it is crucial that the linking numbers of the plasmids are not altered during or after cell lysis. In a control experiment, equal volumes of two exponentially growing NK7048 transformants, one harboring ptacTOP1 and the other a 2.3-kilobase-pair tetracycline-resistant control plasmid pJW301, were mixed after IPTG induction, and the cells were lysed as before. Two-dimensional gel electrophoresis reveals that the distribution of pJW301 topoisomers in the mixture and from cell cultures containing only pJW301 are indistinguishable. Thus, the presence of cells expressing a high level of yeast DNA topoisomerase I in the same lysis mixture does not affect the pJW301 topoisomer distribution, suggesting that the yeast enzyme is rapidly inactivated in the lysis procedure.

DISCUSSION

We have shown that the expression of yeast topoisomerase I in *E. coli* can complement the temperature-sensitive *E. coli* DNA topoisomerase I in strain AS17. The yeast and *E. coli* enzymes differ in their primary structures, substrate preference, and mechanism of catalysis; their only known common feature is their interconversion of DNA topoisomers. The notion that the relaxation activity of the eukaryotic enzyme is responsible for its role in restoring *E. coli* AS17 cell viability is supported by the correlation of the ability of various 5′-deletion mutants of *TOP1* to complement the lethal *topA* phenotype with the reduction in the negative superhelicity of intracellular plasmids expressing these truncated *TOP1* sequences.

Inside *E. coli*, there might be subtle differences in the actions of the eukaryotic and prokaryotic enzymes. *E. coli* DNA topoisomerase I relaxes highly negatively supercoiled DNA very rapidly; it becomes less effective as the degree of negative supercoiling decreases (11). Thus, the enzyme appears to be well-suited to keeping intracellular DNA moderately negatively supercoiled. In contrast, the rate of relaxation by the eukaryotic enzyme, which is independent of

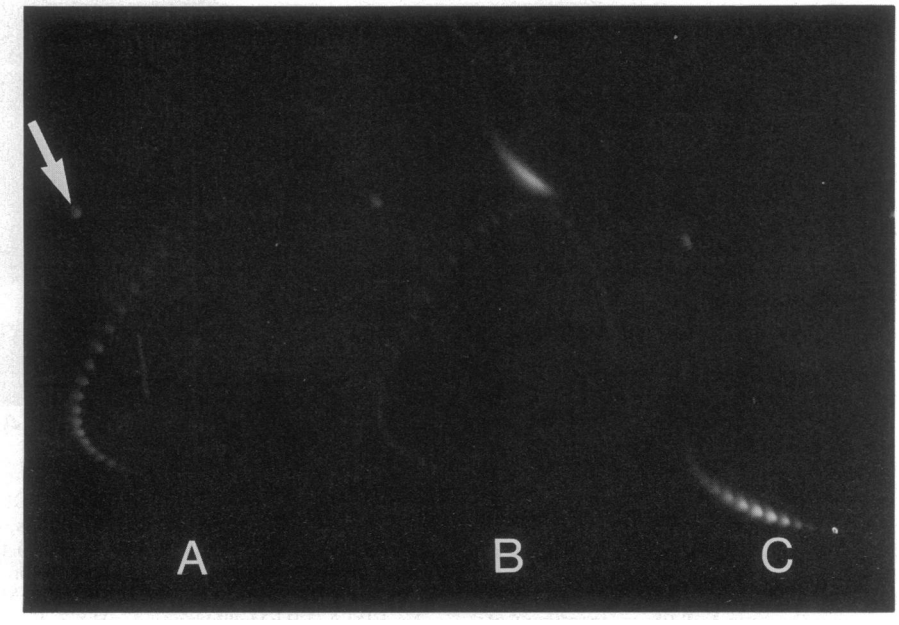

FIG. 3. Two-dimensional gel electrophoresis of ptacTOP1 and ptacTOP1Δ415 topoisomers from IPTG-induced and uninduced cells. Plasmid ptacTOP1 DNA from uninduced cells (*A*), IPTG-induced cells (*B*), and ptacTOP1Δ415 DNA from IPTG-induced cells (*C*) were loaded onto a 0.7% agarose slab gel for two-dimensional electrophoresis. Electrophoresis in the first dimension was for 20 hr in 0.1 M Tris base/0.1 M sodium borate/2 mM Na_2EDTA/1.9 μM chloroquine, pH 8.3. The gel was soaked overnight in the same buffer except the chloroquine concentration was increased to 9.7 μM, rotated 90°, and electrophoresed in the soaking buffer (2.6 V/cm, 17 hr). The DNA was visualized by UV illumination following extensive washing to remove the chloroquine and staining with ethidium bromide.

DNA superhelicity, is not fine-tuned to the degree of supercoiling. A specific instance where differences in topoisomerase activities may be considered is gene transcription. It has been suggested (33) that, under certain conditions, transcription may increase the degree of negative supercoiling of the DNA template behind the advancing polymerase and decrease the degree of negative supercoiling of the DNA template ahead of the polymerase; it is plausible that positively as well as negatively supercoiled regions may form locally. Normally in *E. coli*, only gyrase can relax the positively supercoiled loops. However, in cells expressing yeast DNA topoisomerase I, the yeast enzyme can relax positive as well as negative supercoils.

Although *E. coli* cannot tolerate a high degree of negative supercoiling resulting from inactivation of DNA topoisomerase I, it is unknown if there is a lower limit of supercoiling that is required for viability. The results depicted in Fig. 3 show that induction of the *TOP1* gene in cells harboring ptacTOP1 yields a broad topoisomer distribution with a range of ≈45 in linking number or ≈0.09 in specific-linking difference. Although topoisomers near the high linking-number end of the arc shown in the center of Fig. 3 are close to being completely relaxed, the presence of topoisomers with much lower linking numbers persists. We do not know if these plasmids come from a subpopulation, or if there is a dynamic equilibrium among the different topoisomers. These uncertainties make it difficult to interpret the physiological effects of very low degrees of supercoiling in bacteria and to extrapolate supercoiling studies done with plasmids to the chromosomal DNA (see the discussion in ref. 33 for the differences in supercoiling due to transcription of plasmids and chromosomal DNA loops).

We found that ≈130 amino acids or 18% of the total can be deleted from the amino terminus of yeast DNA topoisomerase I without inactivating *in vitro* activity of the enzyme. This accounts, at least in part, for earlier preparation procedures yielding a 60-kDa eukaryotic DNA topoisomerase I (reviewed in refs. 1 and 2). Some of the amino-terminal deletions are active *in vitro* but inactive inside *E. coli*; it should be of interest to test if the same is true in yeast and if the portions deleted affect the functions of the enzyme.

Finally, we note that a number of antibiotics and antitumor drugs have been found to act by blocking the DNA resealing step in the topoisomerase-catalyzed DNA-strand breakage and rejoining (reviewed in ref. 4). Whereas most of the known drugs act on the type II topoisomerases, the drug camptothecin acts on eukaryotic DNA topoisomerase I (34). The present work establishes that yeast DNA topoisomerase I, and presumably the same enzyme from other eukaryotes including humans, can be expressed in a catalytically active form inside *E. coli*. Such a system may be useful in the screening of drugs acting on eukaryotic DNA topoisomerase I.

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