The Role of the Carboxyl-terminal Amino Acid Residues in Escherichia coli DNA Topoisomerase III-mediated Catalysis*

Hong Liang Zhang†, Swati Malpure§, Zhiyu Li§, Hiroshi Hiasa¶, and Russell J. DiGate‡§ **

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From the Molecular and Cell Biology Program, University of Maryland, Baltimore, Maryland 21201, the Department of Pharmaceutical Sciences, University of Maryland at Baltimore School of Pharmacy, Baltimore, Maryland 21201, the Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, and the Biotechnology Center, Maryland Biotechnology Institute, University of Maryland, Baltimore, Maryland 21201

The role that the carboxyl-terminal amino acids of Escherichia coli DNA topoisomerase I (Topo I) and III (Topo III) play in catalysis was examined by comparing the properties of Topo III with those of a truncated enzyme lacking the generalized DNA binding domain of Topo III, Topo I, and a hybrid topoisomerase polypeptide containing the amino-terminal 605 amino acids of Topo III and the putative generalized DNA binding domain of Topo I. The deletion of the carboxyl-terminal 49 amino acids of Topo III decreases the affinity of the enzyme for its substrate, single-stranded DNA, by approximately 2 orders of magnitude and reduces Topo III-catalyzed relaxation of supercoiled DNA and Topo III-catalyzed resolution of DNA replication intermediates to a similar extent. Fusion of the carboxyl-terminal 312 amino acid residues of Topo I onto the truncated molecule stimulates topoisomerase-catalyzed relaxation 15–20-fold, to a level comparable with that of full-length Topo III. However, topoisomerase-catalyzed resolution of DNA replication intermediates was only stimulated 2–3-fold. Therefore, the carboxyl-terminal amino acids of these topoisomerases constitute a distinct and separable domain, and this domain is intimately involved in determining the catalytic properties of these polypeptides.

Escherichia coli contains two type I DNA topoisomerases, DNA topoisomerase I (Topo I), a 865-amino acid polypeptide (4), has been proposed to be involved with the maintenance of the superhelical density of the bacterial chromosome by acting as an antagonist to DNA gyrase (reviewed by Wang (5)). It has been shown to possess an ATP-independent relaxation activity (1) and is capable of catalyzing the catenation of nicked, duplex DNA circles (6–8) and the decatenation of these singly linked catenanes (6, 9).

DNA topoisomerase III (Topo III), a 653-amino acid polypeptide (10), was originally purified as a superhelical DNA relaxation activity from cells containing a deletion of the gene encoding Topo I (topA) (2, 3). It was subsequently purified as a potent decatenating activity based on its ability to resolve plasmid DNA replication intermediates in vitro (11). Unlike Topo I, Topo III-catalyzed relaxation of negatively supercoiled DNA was virtually undetectable under standard assay conditions (10 mM Mg²⁺, 50 mM Na⁺ or K⁺, 37 °C), requiring high temperature (52 °C) and low magnesium (1 mM) and monovalent salt concentrations (< 20 mM) to exhibit maximal activity (2, 3, 11). The decatenation of multiply interlinked plasmid DNA dimers and resolution of DNA replication intermediates catalyzed by Topo III, however, does not require these extreme conditions and proceeds quite efficiently under the standard reaction conditions stated above (11). Interestingly, Topo I does not catalyze the resolution of DNA replication intermediates in vitro (12). This has led to the suggestion that Topo III may play a role in the decatenation of newly replicated DNA, whereas Topo I is involved in the maintenance of the superhelical density of the chromosome (11).

Analysis of the gene encoding Topo III (topB) indicated that this topoisomerase exhibited striking protein sequence homology to Topo I (10). Topo I and Topo III, therefore, are an example of two proteins, sharing extensive protein sequence homology, that catalyze distinct reactions. The homology between Topo I and Topo III extends only through the first 600 amino acids of the two polypeptides. The carboxyl-terminal amino acid residues of Topo I and Topo III show no homology, but each has been shown to be involved in substrate binding (13, 14). The carboxyl terminus of Topo I contains three zinc finger motifs and a high density of arginine and lysine residues (15). The carboxyl terminus of Topo III does not contain any motif but, similar to Topo I, contains a high density of clustered, positively charged amino acid residues (14). Since the carboxyl-terminal residues differ in the two polypeptides, the role that this region plays in topoisomerase-catalyzed relaxation and decatenation was examined. The nature of the differences in the two polypeptides was addressed by comparing the properties of Topo III with a truncation of the enzyme (lacking the putative carboxyl-terminal 49-amino acid residue substrate binding domain (14)), Topo I, and a hybrid molecule that contained the amino-terminal 605 amino acids of Topo III fused to the carboxyl-terminal 312-amino acid residue substrate binding domain of Topo I.

MATERIALS AND METHODS

DNA and Nucleotides—αX174 RF I DNA was purchased from Life Technologies Inc. DNA oligonucleotides were prepared by the University of Maryland Biopolymer Laboratory. Radiolabeled nucleoside triphosphate was purchased from Amersham Corp. Enzymes and Reagents—Acrylamide and agarose were from Life Technologies, Inc. Bacteriophage T4 polynucleotide kinase was from New England Biolabs Inc. Nucleoside P3 was purchased from Boehringer Mannheim.

Protein Determination—Protein concentration was determined by the method of Bradford (16) using a Bio-Rad protein assay kit.

Radiolabeling of Oligonucleotides—Oligonucleotides were 5’-end-labeled using bacteriophage T4 polynucleotide kinase and [γ-32P]ATP as per the manufacturer’s recommendations. The labeled oligonucleotides...
were fractionated through a polyacrylamide gel. The region containing the labeled oligonucleotide was excised, and the DNA was isolated by direct elution of the fragment into 10 mM Tris-HCl (pH 7.5 at 22°C), 1 mM EDTA. The radiolabeled oligonucleotides were diluted to a specific activity of 2000 cpn/pmol by the addition of excess unlabeled oligonucleotide.

Construction of the Chimeric Topo I-Topo III Gene—The gene encoding the Topo III-Topo I fusion protein was constructed by first introducing a PvuII restriction endonuclease site, using oligonucleotide-directed site-specific mutagenesis (17), into Mpi9 DNA that contained topB (10) (the gene encoding Topo III) and topA (12) (the gene encoding Topo I) that had been engineered to be subcloned into the T7 transient expression vector pET-3c (18). The PvuII endonuclease site was positioned to cleave the topB sequence after the codon specifying amino acid Gin^505 and to cleave the topA sequence after the codon specifying amino acid Gin^652. The Mpi9 DNA (RF I) that contained the altered topB gene was then cleaved with NdeI and PvuII restriction endonucleases to liberate a fragment encoding the first 605 amino acids of Topo III. The NdeI-PvuII fragment was separated by agarose gel electrophoresis, the band was excised from the gel, and the fragment was purified using an IBI electrophoretic apparatus. The Mpi9 DNA (RF I) that contained the cleaved altered topA gene was cleaved with PvuII and BglII restriction endonucleases to liberate a fragment encoding the carboxyl-terminal amino acids (amino acids 553-865) of Topo I (a BglII site was previously placed downstream of topA in order to allow ligation into the BamHI site of pBluescript II plasmid (12)). The PvuII-BglII fragment Topo I was separated by agarose gel electrophoresis, the band was excised from the gel, and it was purified with an IBI electrophoretic apparatus.

The two fragments were then used in a ligation containing NdeI-BamHI cut pET-3c in order to generate the hybrid gene (pT31Z). A plasmid DNA containing the correct insert was isolated and was then transformed in E. coli BL21.

Purification of Topo 31Z, Topo III, Topo I, and Topo III^605—The induction of the chimeric polypeptide (Topo 31Z) was initiated by injection of the expression strain, harboring the pT31Z plasmid DNA, with bacteriophage λ CE6 (18). Induction of the hybrid protein was performed at 30 °C in order to maximize the topoisomerase activity present in the reaction lysate. Chromatography through a trypsin inhibitor agarose column was included in the purification of each polypeptide to reduce proteolysis. (12). In order to prevent any contamination of the hybrid polypeptide with endogenous Topo III, it was purified from E. coli strain BL21 in which the gene encoding Topo III (topB) had been disrupted (12). Both polypeptides were purified by a modification of a previously described protocol that included DE52, Biogel HT, single-stranded DNA cell culture, and Sephacryl S-200 chromatography (11). The purification of Topo III, Topo I, and Topo III^605 has been described previously (12, 14).

Superhelical DNA Relaxation Assays—Superhelical DNA relaxation reaction mixtures (25 μl) contained 40 μl Hepes-KOH buffer (pH 8.0 at 22°C), 1 mM magnesium acetate (pH 7.0), 0.1 mg/ml bovine serum albumin, 40% (v/v) glycerol, 200 ng of X174 RNA, and the indicated amount of topoisomerase (14). The reactions were incubated at 52 °C for 10 min, and the reaction products were separated through an agarose gel and visualized by staining with ethidium bromide as described previously (19).

oriC DNA Replication Assay—The replication of oriC-containing DNA, in vitro, was performed as described previously (12). The replication products were separated by agarose gel electrophoresis and visualized by autoradiography (19). The percentage of replication products existing as Form II molecules was quantified using a Fuji BAS 1000 phosphor imager.

Oligonucleotide Gel Mobility Shift Assays—Reaction mixtures (10 μl) contained 40 μl Hepes-KOH buffer (pH 8.0 at 22°C), 0.1 mg/ml bovine serum albumin, 1 mM magnesium acetate (pH 7.0), 12% glycerol, 5 pmol of radiolabeled oligonucleotide, and the indicated amount of topoisomerase. The reactions were incubated for 5 min at 37°C, and the products were separated through a polyacrylamide gel (30:0.8) using 0.5 × TBE as the running buffer. The gels were electrophoresed at 15 mA for 1.5 h, dried, and autoradiographed. The 45-base radiolabeled oligonucleotide used in this assay was 5'-CAGAGATGGCCGC|TTGTG|TCT|TTG|GGG|TGTGAAGCTCAACAAT-3' (oligonucleotide 45C), where up indicates the site of Topo III cleavage and down indicates the site of Topo I cleavage. The autoradiographs were quantified using a Pharmacia Biotech Inc. Ultrascan laser densitometer. In addition, bands from the gels, representing the indicated topoisomerase-oligonucleotide complex, were excised, and the amount of radiolabeled oligonucleotide was determined using a Beckman LS 5801 liquid scintillation counter.

Topoisomerase-induced DNA Cleavage Assay—Reaction mixtures (5 μl) contained 40 μl Hepes-KOH buffer (pH 8.0 at 22°C), 0.1 mg/ml bovine serum albumin, 1 mM magnesium acetate (pH 7.0), and 5 pmol of a radiolabeled 22-base oligonucleotide. The oligonucleotide was the following subsequence of the 45-base oligonucleotide (45C): GAATGACCCGC|TAC|T|TC|G|GG|G|AT, where up indicates the site of Topo III cleavage and down indicates the site of Topo I cleavage. The indicated amount of Topo III, Topo I, or Topo I, was incubated for 3 min at 37°C, and the reaction was stopped by the addition of SDS to 2%. The reactions were adjusted to 45% formamide, 10 μl EDTA, 0.25% bromphenol blue, 0.25% xylene cyanol, and heat-denatured for 5 min at 90°C. The reaction products were separated by electrophoresis through a polyacrylamide gel (19:1) containing 5% (w/v) urea. The gels were then dried and autoradiographed.

Nuclease P1 Protection Assay—Reaction mixtures (5 μl) contained 40 μl Hepes-KOH buffer (pH 8.0 at 22°C), 0.1 mg/ml bovine serum albumin, 1 mM magnesium acetate (pH 7.0), and 200 fmol of the 45-base radiolabeled oligonucleotide. Reactions containing Topo I and Topo 31Z were incubated for 3 min at 37°C followed by the addition of 3 × 10^3 units of P1 nuclease as described previously (14). The reactions were incubated an additional 10 min at 37°C and terminated by the addition of EDTA to 0.02 M. The reactions were adjusted to 45% formamide, 0.25% bromphenol blue, 0.25% xylene cyanol, and heat-denatured for 5 min at 90°C. The reaction products were separated by electrophoresis through a polyacrylamide gel (19:1) containing 5% (w/v) urea. The gels were then dried and subjected to autoradiography.

RESULTS

Overproduction and Purification of Topo 31Z, a Topoisomerase I-Chimera—In order to examine what was responsible for the different catalytic properties of Topo I and Topo III, a chimeric molecule, Topo 31Z, was synthesized (Fig. 1A) that combined the first 605 amino acid residues of Topo III (which are homologous to those of Topo I) with the carboxyl-terminal amino acid residues of Topo I (which show no homology with Topo III). The chimeric gene was engineered in such a way that the only significant change between the two enzymes was the replacement of valine 553 of Topo I with a leucine residue (Fig. 1B). This Topo III-Topo I hybrid gene was

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The enzyme's ability to bind its substrate. In order to evaluate this hypothesis, oligonucleotide mobility shift experiments were performed using a 45-base oligonucleotide (14), containing both Topo I and Topo III cleavage sites, as a substrate (Fig. 4). Topo III, Topo III604, Topo 31Z, and Topo I were evaluated for their ability to form a stable complex with the 45-base oligonucleotide (oligonucleotide 45C). In accord with previous results (14), the truncation of Topo III to 604 amino acid residues dramatically decreases equilibrium binding of the enzyme to its substrate. In fact, no oligonucleotide-topoisomerase complex is observed with the levels of the truncated enzyme used in this experiment (lanes 5–7). The addition of the carboxyl-terminal residues of Topo I to the truncated enzyme, however, stimulates equilibrium binding of the enzyme to its substrate. Topo 31Z (lanes 8–10) is approximately 11% as efficient as Topo III (lanes 2–5) and Topo I (lanes 11–13) in binding to oligonucleotide 45C. Previous studies have indicated that the equilibrium binding of Topo III604 is approximately 0.5% of the full-length polypeptide (14); therefore, the addition of the carboxyl-terminal amino acids of Topo I stimulates equilibrium binding of Topo III604 by 20-fold. This is in excellent agreement with stimulation observed for Topo 31Z in the relaxation of negatively supercoiled DNA substrates.

The cleavage site specificity of Topo 31Z was also compared with Topo III and Topo I (Fig. 5). A 22-base oligonucleotide containing a subsequence of the 45-base oligonucleotide was used as a substrate in a topoisomerase-catalyzed DNA cleavage assay. This oligonucleotide contains distinct Topo III and Topo I cleavage sites. Topo 31Z-induced cleavage of this substrate occurs at the strong Topo III site (compare lanes 1 and 2 to lane 4) rather than the Topo I cleavage sites (lane 3). This is consistent with the observation that the requirements for the relaxation of supercoiled DNA by the hybrid molecule closely

![Fig. 2. SDS-polyacrylamide gel electrophoresis of purified Topo 31Z. Topo 31Z was purified to apparent homogeneity as described under "Materials and Methods." 1.0 μg of the purified enzyme was electrophoresed through a 10% polyacrylamide in the presence of 0.1% SDS (27). The protein was visualized by staining the gel with Comassie Blue. Lane 1, Topo 31Z; lane 2, molecular mass markers.](Image 1)

![Fig. 3. DNA relaxation assay of Topo III, Topo III604, Topo I, and Topo 31Z. DNA relaxation assays were performed as described under "Materials and Methods." Upper panel, reactions (25 μl) contained no topoisomerase (lanes 1 and 10), 200 fmol (lane 2), 500 fmol (lane 3), 1000 fmol (lane 4), or 1500 fmol (lane 5) of Topo III604 or 50 fmol (lane 6), 100 fmol (lane 7), 200 fmol (lane 8), or 500 fmol (lane 9) of Topo 31Z. Lower panel, reactions (25 μl) contained no topoisomerase (lanes 1 and 14) or 50 fmol (lanes 2, 6, and 10), 100 fmol (lanes 3, 7, and 11), 200 fmol (lanes 4, 8, and 12), and 500 fmol (lanes 5, 9, and 13) of either Topo 31Z (lanes 2–5), Topo I (lanes 6–9), or Topo III (lanes 10–13). OC, open circle (nicked or gapped circular DNA); SC, supercoiled (negatively supercoiled circular DNA).](Image 2)
were never observed. The lack of fully decatenated plasmid monomers may be a consequence of the inhibition of the replication reaction by increasing amounts of Topo I (Fig. 6A, lanes 1-5). It has been shown that the replication reaction can be inhibited by relaxation of the input template by Topo I (20, 21). It is not clear whether Topo I is incapable of fully decatenating the replication intermediates or simply if the amount of Topo I required to generate fully decatenated products results in the inhibition of the replication reaction. Topo III, however, was a potent decatenase and readily resolved the plasmid DNA replication intermediates (Fig. 6A, lanes 7-10).

Interestingly, in contrast to the 15-20-fold stimulation of DNA relaxation activity compared with that of the truncated enzyme, the addition of the carboxyl-terminal amino acid residues of Topo I to the truncated enzyme (Topo 31Z) had only a minimal effect on the resolution of DNA replication intermediates (Fig. 6B, lanes 2-5). The hybrid enzyme showed, at most, a 2-3-fold stimulation of decatenation activity when compared with the 604-amino acid truncation of Topo III (Fig. 6B, lanes 7-10). Topo III604 is 1-2% as active as the full-length polypeptide, consistent with the DNA relaxation activity exhibited by the enzyme.

In contrast to Topo I, however, Topo 31Z was capable of completely resolving plasmid DNA replication intermediates (albeit at a reduced efficiency). This may represent an intrinsic and unique property of the first 605 amino acids of Topo III.

The Presence of the Carboxyl-terminal Residues of Topo I in Topo 31Z Alters the Substrate Binding Properties of Topo III—Although the restoration of substrate binding efficiency by the carboxyl-terminal residues of Topo I could account for the stimulation of Topo 31Z-catalyzed DNA relaxation activity (Fig. 3), it could not account for the significantly reduced stimulation of the resolution of DNA replication intermediates (Fig. 6). Therefore, the binding properties of the hybrid enzyme were further characterized by a nuclease protection experiment using both Topo I and Topo 31Z (Fig. 7). Topo III has been shown to be a site-specific binding protein that protects a 14-base region surrounding its cleavage site (22). In contrast to Topo III (Fig. 7A, lane 1), a titration of Topo 31Z (Fig. 7A, lanes 2-5) and Topo I (Fig. 7B, lanes 1-4) revealed no distinct nuclease protection pattern. This observation suggests either that a single molecule of Topo I or Topo 31Z protects the entire oligonucleotide or that the topoisomerase molecules bind along the length of the entire oligonucleotide. It is clear, however, that the binding properties of the enzyme have been altered, and the protection pattern generated by Topo 31Z is similar to that generated by Topo I. This change in the binding properties of the hybrid molecule may account for the altered properties of the enzyme.

**DISCUSSION**

The carboxyl-terminal residues of Topo I and Topo III are required for the formation of a stable enzyme-substrate complex, and it has been postulated that this region may constitute a generalized DNA binding domain (13, 14). Since this domain lies outside the region of homology between the two polypeptides, the possibility that the distinct reactions catalyzed by the two enzymes were the result of the properties of their heterologous carboxyl-terminal domains was examined. This was accomplished by determining the biochemical properties of a chimeric enzyme in which the carboxyl-terminal residues of Topo I were substituted for those of Topo III.

A 604-amino acid truncation of Topo III (Topo III604) had been shown to possess a very low affinity for single-stranded DNA when compared with the full-length molecule (14). The protein sequence homology between Topo I and Topo III breaks around this point in the amino acid sequence comparison of the two enzymes; therefore, this region was chosen as the site for

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**Fig. 4.** Oligonucleotide gel mobility shift assay of Topo III, Topo III604, Topo I, and Topo 31Z. A 45-base oligonucleotide that contained a strong Topo III binding/cleavage site (oligonucleotide 45C) was incubated with no topoisomerase (lane 1), or 1.2 pmol (lanes 2, 5, 8, and 11), 3.6 pmol (lanes 3, 6, 9, and 12), or 10.8 pmol (lanes 4, 7, 10, and 13) of Topo III (lanes 2-4), Topo III604 (lanes 5-7), Topo 31Z (lanes 8-10), or Topo I (lanes 11-13). The reactions were processed and resolved through a 10% polyacrylamide gel. The position of the stable topoisomerase-oligonucleotide complex is indicated. The amount of the topoisomerase-oligonucleotide complex was determined as described under "Materials and Methods."

**Fig. 5.** Determination of the cleavage site specificity of Topo 31Z. Cleavage reactions were performed as described under "Materials and Methods." Reactions contained 1 pmol (lane 1) or 0.1 pmol (lane 2) of Topo 31Z, 0.1 pmol of Topo I, or 0.1 pmol of Topo III. Reaction products were separated by electrophoresis through a 25% polyacrylamide gel in the presence of 50% (w/v) urea as described under "Materials and Methods." NT, nucleotides.
splicing the carboxyl-terminal amino acid residues of Topo I to Topo III. The hybrid enzyme was overexpressed, purified, and assayed for supercoiled DNA relaxation activity and its ability to resolve DNA replication intermediates in vitro.

The addition of the carboxyl-terminal amino acids of Topo I to Topo III,604 was able to restore the ability of the enzyme to resolve DNA replication intermediates assayed for supercoiled DNA relaxation activity and its ability to bind to single-stranded DNA. The hybrid enzyme had a similar ability to bind to single-stranded DNA as the truncated enzyme. The hybrid enzyme was overexpressed, purified, and assayed for supercoiled DNA relaxation activity and its ability to bind to single-stranded DNA. The hybrid enzyme had a similar ability to bind to single-stranded DNA as the truncated enzyme.

An analysis of binding specificity of both Topo I and Topo 31Z indicated that these enzymes bind and protect the entire 45-base oligonucleotide substrate. This result is distinctively different from the 14-base protection pattern exhibited by Topo III (22). Therefore, it is clear that the substitution of the generalized binding domain of Topo I for that of Topo III alters the manner in which Topo III binds to its substrate and alters the catalytic properties of the enzyme. A striking difference between Topo I and Topo III is that in addition to a preference for single-stranded substrates, Topo I also has a considerable affinity for double-stranded DNA (23). In contrast, only single-stranded DNA appears to be an effective substrate for Topo III binding (11, 14).

A model for the mechanism of both decatenation and relaxation, based on the properties of the generalized binding domains of Topo I and Topo III, data from other laboratories, and from the known three-dimensional structure of Topo I is presented in Fig. 8. The mechanism of topoisomerase-induced decatenation is based on the model by Mondragon and colleagues (24). In this model (Fig. 8A), one helix (represented by the circle) is located in the cavity of the inverted "U" structure of the topoisomerase. The generalized DNA binding domain (represented by the rectangle) is positioned asymmetrically across the body of the topoisomerase. This domain is responsible for noncovalent interactions with the substrate 5' to the topoisomerase cleavage site (22). Decatenation occurs when a tyrosine residue in the active site of the enzyme (triangle) transiently nicks the single-stranded DNA, creating a "gate" that allows the helix located in the cavity of the enzyme to pass.

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through the nick. Since the generalized DNA binding domain of Topo III binds single-stranded DNA, the enzyme is always capable of passing a helix through the gate and, hence, always capable of decatenating the two molecules. The generalized DNA binding domain of Topo I, however, has a significant affinity for double-stranded DNA in addition to its affinity for single-stranded DNA (23). If double-stranded DNA were present in the enzyme active site, the gate would be blocked by the uncleaved strand of DNA since a type I topoisomerase can only cleave one strand of the substrate. The same result would occur if the enzyme bound to a region of a single-stranded substrate that did not contain a Topo I cleavage site. These binding properties would result in an enzyme that is inefficient at decatenation. This would also explain the inefficiency observed in Topo III-catalyzed decatenation since, the majority of the time, the enzyme would be presented with a substrate that is refractory to decatenation.

Relaxation of negatively supercoiled DNA (Fig. 8B) is accomplished by the binding of double-stranded DNA to the generalized DNA binding domain of Topo I in a mechanism analogous to the bridging model originally proposed by Cozzarelli and colleagues (7, 26). In this case, the single-stranded region required for cleavage need only be present locally around the active site tyrosine (triangle) of the enzyme. The three-dimensional structure of Topo I indicates that there is a cleft capable of fitting single strand DNA in the vicinity of the active site of the enzyme (24). The generalized binding domain may simply feed the double-stranded molecule into this channel resulting in the local denaturation of the molecule into two single strands (this denaturation step is greatly stimulated by negative superhelicity). Relaxation occurs by the transient cleavage of one of the strands (as described above) followed by the strand passage of the opposite strand (shown in boldface) through the gate. This energy is provided by potential energy stored in the form of superhelicity within the molecule. Decatenation could not be accomplished even if a helix were present in the cavity of the enzyme since the uncleaved strand of DNA would serve to prevent strand passage. A more detailed description is given under "Discussion."
motes the creation of a node and/or stabilizes this intermediate.

The crystal structure of the amino-terminal 596 residues of Topo I has been determined to 2.2-Å resolution (24). Unfortunately, this structure does not contain the carboxyl-terminal substrate binding domain. The structure of carboxyl-terminal domain of Topo I has been determined using multidimensional NMR methods (25); however, since this structure was obtained from a purified carboxyl-terminal peptide, it is unclear how this structure relates to the known crystal structure of the enzyme. However, crystals have been obtained of the full-length Topo III polypeptide. In addition, the availability of a catalytically inactive Topo III polypeptide that has the same binding specificity as the active enzyme (22) should allow the structural determination of a Topo III-substrate complex.

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A. Mondragon, personal communication.