Escherichia coli Type-1 Topoisomerases: Identification, Mechanism, and Role in Recombination


Access the most recent version at doi:10.1101/SQB.1983.047.01.088

References
This article cites 45 articles, 19 of which can be accessed free at:
http://symposium.cshlp.org/content/47/769.refs.html

Article cited in:
http://symposium.cshlp.org/content/47/769#related-urls

Email alerting service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.

To subscribe to Cold Spring Harbor Symposia on Quantitative Biology go to:
http://symposium.cshlp.org/subscriptions

Copyright © 1983 Cold Spring Harbor Laboratory Press
Escherichia coli Type-1 Topoisomerases: Identification, Mechanism, and Role in Recombination

F. DEAN,* M.A. KRASNOW,* R. OTTER,* M.M. MATZUK,† S.J. SPENGLER,† AND N.R. COZZARELLI†

*Department of Biochemistry, University of Chicago, Chicago, Illinois 60637; †Department of Molecular Biology, University of California, Berkeley, California 94720

Topoisomerases catalyze the concerted breakage and reunion of DNA strands. The energy of the broken phosphodiester bond is conserved in a covalent linkage of the enzyme to DNA, allowing the break to be resealed in the absence of an external energy source. Topoisomerases carefully control the breakage and reunion process, never leaving the DNA while it is broken. These enzymes carry out diverse reactions including supercoiling, catenation, knotting, recombination, and aspects of DNA replication. Type-2 topoisomerases make a transient double-stranded break and pass duplex DNA through the break. This changes the topological linking number of DNA ($L_k$) in steps of two, as explained by the sign-inversion mechanism (Brown and Cozzarelli 1979; Liu et al. 1980; Gellert 1981). Type-1 topoisomerases, on the other hand, break only one strand at a time and alter the $L_k$ in steps of one (Champoux 1978; Wang and Liu 1979; Cozzarelli 1980b).

In this paper we focus on the type-1 topoisomerases of Escherichia coli, enzymes that have recently received increased attention. Five topics are considered. First, in the analysis of the electrophoretic mobility of negatively supercoiled topoisomers, we discovered that adjacent DNA bands on a gel need not differ by one in linking number. Second, we identified a new Es. coli topoisomerase, topoisomerase III. Third, the mechanism of catenation of duplex DNA rings by Es. coli topoisomerase I has been elucidated. Fourth, we describe the involvement of a type-1 topoisomerase, the resolvase of Tn3, in site-specific recombination. In catenation and recombination by type-I topoisomerases, duplex DNA is topologically rearranged via the breakage and rejoining of DNA a single strand at a time. Fifth, we consider what cellular processes might be particularly suited for the involvement of type-1, but not type-2, topoisomerases.

Assay of Topoisomerases and the Relationship of Linking Number to Electrophoretic Mobility

Topoisomerases are usually assayed by the relaxation of supercoils in a plasmid, as measured by the production of topoisomers with reduced electrophoretic mobility (Crick et al. 1979). However, individual topoisomers of native plasmids in the size range usually employed (4–6 kb) are not well resolved by agarose gel electrophoresis, and only a large reduction in super-twist density can be detected. For a sensitive assay of relaxation, we have used the 1683-bp plasmid, pAO3, and a composite acrylamide-agarose gel (Peacock and Dingman 1968). Nearly all of the topoisomers from fully relaxed to native are resolved well by a single gel, and thus a small change in topoisomer distribution can be discerned.

Using this high-resolution system, we discovered that neighboring electrophoretic bands do not always differ in $L_k$ by one. As the $L_k$ of pAO3 DNA is decreased, topoisomer mobility initially increases, as expected. However, as the number of titratable negative supercoils increases beyond seven, there is a reduction in electrophoretic mobility (Fig. 1). The reduction is not a general consequence of an increase in superhelical density beyond a critical point, because as greater numbers of positive supercoils are introduced, electrophoretic mobility continues to rise (Fig. 1). We suggest instead that increased underwinding stabilizes a DNA structure such as a cruciform (Lilley 1980; Panayotatos and Wells 1981). Since extrusion of a DNA segment into a cruciform reduces the length of the DNA in the circle while...
the $L_k$ remains constant, the negative superhelical density of the DNA in the circle drops. The DNA is then less compact and migrates more slowly than a topoisomerase that is less underwound.

**A New E. coli Topoisomerase**

The properties of the three previously identified *E. coli* topoisomerases are summarized in Table 1. Topoisomerase I ($\omega$ protein) is a 101,000-dalton polypeptide encoded by *topA* (Wang and Liu 1979; Sternglanz et al. 1981; Trucksis and Depew 1981). It is a type-1 enzyme that relaxes negatively supercoiled DNA. Topoisomerase II (DNA gyrase) is a type-2 enzyme that introduces negative supercoils into DNA at the expense of ATP and slowly removes them in the absence of ATP encoded by *gyrA, gyrB* (Cozzarelli 1980a). The subunit encoded by *gyrA* and *gyrB* is responsible for ATP binding and is the target for novobiocin. Topoisomerase II' (Brown et al. 1979; Gellert et al. 1979) contains the 77,000-dalton, that comprise 80% of the preparation. The ratio of the two varies in separate preparations. They are the only polypeptides coincident with activity when the enzyme is analyzed by velocity sedimentation. The identity of their partial proteolysis patterns (Cleveland et al. 1977) is good evidence that they are encoded by a single gene and differ only in processing. The sedimentation value of 5.0S is consistent with a molecular weight of about 65,000 (Martin and Ames 1961), and thus topoisomerase III very likely consists of a single polypeptide of about 75,000 daltons.

Several characteristics distinguish topoisomerase III from topoisomerase I. First, antibodies to topoisomerase I (Trucksis and Depew 1981) do not inhibit topoisomerase III. Second, topoisomerase III is present in a *topA* deletion strain, DM800 (Sternglanz et al. 1981), that has no detectable topoisomerase I activity. DM800 is a convenient strain for the purification of topoisomerase III because the two enzymes behave similarly in several purification steps. Third, the partial proteolysis patterns of the topoisomerases are entirely different. Fourth, the two enzymes have different sequence specificity. Incubation of topoisomerase I or III with DNA in the absence of Mg$^{++}$, followed by addition of SDS, resulted in cleavage of the DNA and attachment of the enzyme to the resulting broken end (Liu and Wang 1979). The cleavage sites were mapped using single-stranded [5'-32P]DNA. Display of the cleavage products by polyacrylamide gel electrophoresis showed no common cut sites (Fig. 2). Thirty topoisomerase I cleavage sites on single-stranded DNA have been sequenced (Tse et al. 1980; F. Dean and N. Cozzarelli, unpubl.). All have a cytosine residue four nucleotides to the 5' side of the break (Fig. 3). The breaks produced by topoisomerase III lack cytosine in this position. Topoisomerase III is clearly distinguished from topoisomerases II and II' on the basis that it is type 1, insen-

**Figure 2.** Single-stranded DNA cleavage specificity of topoisomerases I and III. The substrate was a 687-base fragment of single-stranded DNA prepared from ϕX DNA. Reactions (10 µl) contained 30% glycerol, 20 mM Tris-HCl (pH 7.6), 20 mM KCl, 0.5 mM dithiothreitol, 50 µg/ml of serum albumin, [5'-32P]DNA, and 15 ng of topoisomerase I or 6 ng of topoisomerase III. After 25 min at 52°C, SDS was added to 1%. Samples were analyzed by polyacrylamide (12.5%) gel electrophoresis (Maxam and Gilbert 1980).

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Gene</th>
<th>Protomer molecular weight ($\times 10^4$)</th>
<th>Type</th>
<th>Relaxes positive supercoils</th>
<th>Inhibited by</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (ω)</td>
<td>$\alpha_1$</td>
<td><em>topA</em></td>
<td>101</td>
<td>1</td>
<td>no</td>
<td>–</td>
</tr>
<tr>
<td>II (gyrase)</td>
<td>$\alpha_2\beta_2$</td>
<td><em>gyrA, gyrB</em></td>
<td>105; 95</td>
<td>2</td>
<td>no</td>
<td>+</td>
</tr>
<tr>
<td>II'</td>
<td>$\alpha_1\beta_1$</td>
<td><em>gyrA, gyrB?</em></td>
<td>105; 50</td>
<td>2</td>
<td>yes</td>
<td>+</td>
</tr>
<tr>
<td>III</td>
<td>$\alpha_1$</td>
<td><em>topR</em></td>
<td>75</td>
<td>1</td>
<td>?</td>
<td>–</td>
</tr>
</tbody>
</table>

The $\alpha$ and $\beta$ designations for protomer composition do not indicate genetic relatedness (shown in a separate column). The structures indicated for topoisomerase III and resolvase are likely, but not as rigorously demonstrated as for the other enzymes. Only *E. coli* strains that harbor Tn3, γ5, or related transposons have resolvase. Excluded from the table are phage-encoded topoisomerases. Nal and Novo indicate nalidixic acid and novobiocin, respectively.
E. **COLI** TYPE-1 TOPOISOMERASES

---

**Figure 3.** Topoisomerase I cleavage sequences for single-stranded DNA. The incidence of nucleotides around 30 topoisomerase I cleavage sites (Tse et al. 1980; F. Dean and N. Cozzarelli, unpublished) are compiled. The common attribute is the presence of a cytosine residue four nucleotides to the 5' side of the cut site.

---

Mechanism of Type-I Topoisomerases

The discovery of catenation and knotting of intact duplex DNA rings provided strong evidence for the sign-inversion model for type-2 topoisomerases because these reactions require the passage of duplex DNA segments through each other (Brown and Cozzarelli 1979; Liu et al. 1980). The simple connection of these reactions to type-2 topoisomerases was unsettling by the discovery that type-1 enzymes could also catenate and knot DNA (Tse and Wang 1980; Brown and Cozzarelli 1981). The resolution of this paradox comes from a consideration of DNA substrate specificity. Catenation requires a donor circle and a recipient circle that is transiently broken by the enzyme. For type-1 enzymes, the recipient must be nicked, whereas the donor can be supercoiled, nicked, or relaxed (Tse and Wang 1980; Brown and Cozzarelli 1981). Neither specificity is expected for topoisomerase I, which binds preferentially to negatively supercoiled DNA (Liu and Wang 1979). However, a simple scheme explains these requirements (see Fig. 4). We postulate that a type-1 topoisomerase introduces a transient single-stranded break opposite the nick in the recipient ring. The donor ring passes through the resultant double-strand interruption; resealing the nick forms a catenane. We demonstrate below that the postulated operation of a type-1 enzyme opposite a nick is correct.

Topoisomerase I has a higher affinity for singly nicked plasmid DNA than for intact DNA (Fig. 5). Fivefold more enzyme is required to bind 1 fmole of intact DNA to a nitrocellulose filter than is required to bind 1 fmole of nicked molecules. Thus, a single nick in a 6.6-kb DNA creates an opportunity for a novel interaction with topoisomerase I.

The binding to a nick cannot be a kinetic dead end because it directs cleavage to the region. An 1108-bp fragment of φX174 DNA was prepared with 32P at the 5' end of the viral (+) strand and a single nick in the...
complementary (−) strand 70 bases from the labeled end (Fig. 6). Cleavages made in the viral strand by topoisomerase I were displayed by gel electrophoresis (see Fig. 7). The exact position of the break was determined by comparison with the mobility of standards produced by chemical cleavage (Maxam and Gilbert 1980) and by restriction enzyme digestion. Only one cleavage was seen and it was three nucleotides from the nick. When the substrate was denatured prior to treatment with topoisomerase I, at least 20 cleavage fragments were produced, including the one seen with the nicked duplex fragment. Unnicked duplex DNA was not cleaved under these conditions.

Under more vigorous cleavage conditions, other breaks are made by topoisomerase I (Fig. 8), but all still cluster in the area of the nick. The nick does not have a rigid directing effect on cleavage, and thus the two breaks do not possess a unique stagger as they do with gyrase cleavage (Morrison and Cozzarelli 1979). In every case a cytosine residue is four nucleotides to the 5' side of the break. Since the same sequence specificity is observed with single-stranded DNA and nicked DNA, we conclude that the enzyme recognizes a similar conformation in each case. The strong binding affinity of topoisomerase I for negatively supercoiled DNA is likely also due to its preference for an unwound region.

We have suggested a model for relaxation of negative supercoils by topoisomerase I (Brown and Cozzarelli 1981) that is consistent with the data on binding and catenation (see Fig. 9). After the unpairing of the strands by enzyme binding, one strand can be passed through a transient break in the other. This will change the Lk in steps of one. The cleaved strand and the passing strand are not base-paired in this one-strand sign-inversion mechanism. This is necessary because catenation is unaffected by sequence complementarity. In fact, topoisomerase I has little specificity for the DNA segment that is passed through a break; it allows a single strand in the process of relaxation, but it can pass nicked, relaxed, or supercoiled duplex DNA in catenation.
**E. coli** Type-1 Topoisomerases

Figure 9. A one-strand sign-inversion mechanism for type-1 topoisomerases. Topoisomerase I binds the DNA such that the double helix is unwound and a node is formed by the crossing of the two single strands. The enzyme transiently nicks one strand, remains bound to each broken end, and passes the other strand through the break. Sealing the break results in a change in linking fixed at +1.

Role of a Type-1 Topoisomerase in Recombination

A more complex case of controlled breakage and reunion occurs in recombination where the segments flanking a pair of double-strand breaks are rearranged before rejoining. There is evidence that a topoisomerase can be responsible for this central event in recombination. The bacteriophage λ Int protein is necessary for the site-specific recombination that mediates the integration and excision of λ DNA from the *E. coli* chromosome. Int is a true topoisomerase since it removes supercoils from circular plasmid DNA (Kikachi and Nash 1979).

We have begun a study of the breakage and reunion reaction of a site-specific recombination involved in DNA transposition. This is part of our continuing study (Fennewald et al. 1981) of the mechanism of transposition of the *E. coli* transposon, Tn3 (Heffron 1982). There are two steps in the transposition of Tn3 from one replicon to another (Fig. 10) (Shapiro 1979). The first is the complex event in which the transposon is duplicated and the replicons are fused via directly repeated transposon bridges. Cointegrate formation requires the product of a single Tn3 gene, *tnpA*, and unidentified host factors. The second step is a site-specific recombination between the transposons that regenerates the two replicons, each now containing Tn3. The second reaction has been shown for the related transposon, γδ, to require only the product of the *tnpR* gene, resolvase (Reed 1981; Reed and Grindley 1981).

The *tnpA*- and *tnpR*-gene products of Tn3 were cloned under the control of the λ *P*₃ promoter and cl repressor as illustrated in Figure 11. This allows the induction of

(Figures and text continue...
Figure 12. Time course of DNA recombination and relaxation by resolvase. pRR51 (1.8 µg), a 5750-bp plasmid containing two directly repeated copies of the resolution site (Reed 1981), was incubated with 50 ng of purified Tn3 resolvase at 37°C in a 192-µl reaction mixture containing 50 mM NaCl, 20 mM Tris-HCl (pH 7.45), 10 mM MgCl₂, and 1 mM dithiothreitol. Samples (16 µl) were removed at the times indicated and either quenched with 0.4% SDS to monitor relaxation (top) or first treated with BamHI nuclease to monitor recombination (bottom). The products were electrophoresed through 1% agarose gels. The two resolution sites in pRR51 are shown as open and closed arrows for ease of visualization, and the unique BamHI site is indicated by an open triangle. BamHI linearizes only the smaller of the rings of the product catenane because there are no BamHI sites in the larger daughter ring.

large quantities of these proteins, and hundreds of milligrams of resolvase have been prepared (M.A. Krasnow et al., unpubl.).

The Tn3 resolvase, like its γδ counterpart (Reed 1981), effectively converts a plasmid containing two directly repeated sites for recombination, called res, into a catenane of the two product rings (Fig. 12). Scission at the unique BamHI nuclease site disrupts the catenane but leaves one product ring circular. Since this ring is nearly fully supercoiled, resolvase maintains close control of the broken ends during the recombination.

Resolvase was shown to be a topoisomerase by its removal of supercoils from the substrate (Fig. 12). It changes the Lk in steps of one and is therefore a type-1 topoisomerase; a comparison with other E. coli topoisomerases is shown in Table 1. The time course for the relaxation and recombination by resolvase was the same (Fig. 12). A more important correspondence in the two processes is that like recombination, relaxation required res sites in the same orientation (Fig. 13). A plasmid with no sites, one site, or two sites in head-to-head orientation was not relaxed at all. Three conclusions can be drawn from this experiment: First, the topoisomerase activity must be intrinsic to the breakage and reunion that marks recombination, because both have the same unique substrate requirements. Relaxation of DNA by Int did not show any sequence preference (Kikuchi and Nash 1979), and therefore the role of its topoisomerase activity in recombination was inferential. Second, resolvase must have at least two active sites, both of which have to be occupied for either to break and rejoin DNA. Third, the two res sites must be brought together in a fixed orientation so that the direct and inverted configuration can be distinguished.

A mechanism for this distinction was suggested by an analysis of the interlinking complexity of the catenated product circles. Using ribbon models for DNA, it can be shown that intramolecular recombination can convert the supercoils of the substrate into intertwining of the product-catenated rings, if the supercoils are plec-
The search for the second res site will disentangle the supercoils such that substrate supercoiling will not affect the number of interwinnings of the product rings. Interlinking would instead be dictated by the mechanism of the enzyme, and the products need not be catenated at all. The tracking between the res sites also explains how the enzyme distinguishes the orientation of the res sites and thereby operates only when they are in the same orientation. If instead the second subunit released DNA and rebound in either orientation, then it would not distinguish the relative orientation of res sites.

Reactions of Type-1 Topoisomerases

Type-2 topoisomerases catalyze the passage of duplex DNA segments through each other and can therefore reversibly catenate and knot intact duplex DNA rings. Because type-1 enzymes make only transient single-stranded breaks, they can (with the exception of site-specific recombination enzymes) only knot and catenate rings that have a preexisting break. They seem, however, particularly suited to a number of reactions (Fig. 16). At the termination of replication of circular DNA, there are topological problems (Gefter 1975) that can be solved by conversion of the incomplete rings to catenanes (Sundin and Varshavsky 1980), and these catenanes could be separated by type-1 enzymes (Fig. 16a). The replication of duplex DNA in which the two strands are covalently joined by a terminal hairpin, such as vaccinia DNA (Geshelin and Berns 1974; Baroudy et al. 1982), could be via single-strand catenanes in which the hairpins are intertwined (Fig. 16b). Separation requires a topoisomerase that operates on single strands. Recombination generally proceeds through a Holliday-structure intermediate (Dressler and Potter 1982) in which duplex DNA segments are joined covalently through two of the four strands (Fig. 16c). Both the formation and resolution of such structures could be by a type-1 topoisomerase such as resolvase. Another process im-

Figure 14. Intramolecular recombination of a supercoiled substrate can lead passively to catenation of the product rings. The two resolution sites are shown as open and closed arrows. Synapsis is the parallel alignment of the resolution sites. Upon recombination, the interwound supercoils trapped between the recombination sites can be directly converted to interlinkings of the daughters. If \( n \) is the number of supercoils and \( M_e \) is the twist lost during breakage and reunion, then \( (n-M_e)/2 \) interlockings of product catenanes can result.

Figure 15. Tracking model for resolvase-mediated recombination. A resolvase dimer (stippled area) binds DNA nonspecifically and translocates randomly along the DNA until reaching a res site (arrow) where it binds tightly and specifically. The second subunit then binds nonspecifically to adjacent DNA, trapping a loop of DNA. One-dimensional diffusion of the second subunit with respect to the DNA causes the loop to expand and contract; resolvase serves as a loose annulus. This segregates the supercoils and prevents net interwinding of the DNA segments between the sites. When the second subunit reaches the second res site in the proper orientation, it is tightly bound, and the enzyme changes conformation, activating breakage and reunion. Strand exchange is directed by resolvase with a specific geometry, resulting in singly interlinked recombinants.
Complicated in recombination is the transfer of a single-stranded segment from one duplex to another (Radding 1978) (Fig. 16d). Single-strand passage by a type-1 enzyme would solve the topological problems of such an intermolecular helix. This is an interpretation of the facilitation of recA-protein-mediated strand transfer by E. coli topoisomerase I (Cunningham et al. 1981). This winding problem also occurs in the annealing of complementary single-stranded circles and this reaction can be carried out with type-1 enzymes (Champoux 1977; Kirkegaard and Wang 1978). The 6X cistron-A protein and the related gene-2 protein of filamentous phages (Kornberg 1980) are examples of still another topoisomerase-mediated process (Fig. 16e). Here, the single-strand breakage and reunion reactions are temporarily interrupted by synthesis of a single-stranded DNA circle. These enzymes relax supercoils and are therefore topoisomerases (Geider and Meyer 1979; Langeveld et al. 1980). Thus, type-1 topoisomerases promote a variety of DNA manipulations that allow facile participation of single strands of DNA in cellular processes.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health research grants GM-21397 and GM-22729 from the National Institute of General Medical Sciences. F.D., M.A.K., and S.J.S. were supported by National Institutes of Health National Research Service Awards; F.D., by grant HD-07136 from the National Institutes of Child Health and Development; M.A.K., by grant GM-07281 from the National Institutes of General Medical Sciences; and S.J.S., by grant CA-09273 from the National Cancer Institute.

REFERENCES


CLEVELAND, D.W., S.G. FISCHER, M.W. KIRSCHNER, and
E. COILI TYPE-1 TOPOISOMERASES


