Contrasting Enzymatic Activities of Topoisomerase IV and DNA Gyrase from *Escherichia coli*

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DNA gyrase and topoisomerase IV (Topo IV) have distinct roles as unlinking enzymes during DNA replication despite 40% sequence identity between them. DNA gyrase unlinks replicating DNA by introducing negative supercoils while Topo IV decatenates the two daughter molecules. For this study, we measured the rates of unlinking of various topoisomers of DNA by DNA gyrase and Topo IV. Each enzyme has marked preferences for certain strand-passage reactions. DNA gyrase is a relatively poor decatenase, catalyzing strand-passage events that result in supercoiling at rates several orders of magnitude faster than those causing decatenation. Topo IV, in contrast, decatenates linked circles 10–40 times more quickly than it removes the intramolecular crossings from supercoiled DNA. Supercoiled catenanes are unlinked at an even more increased rate by Topo IV. Thus, the supercoils augment decatenation rather than compete with catenane crossings for their removal. Knot crossings and the crossings of multiply interlinked catenanes are also preferentially removed by Topo IV. This ability of Topo IV to selectively unlink catenated molecules mirrors its key role in decatenation of replicating chromosomes in *vivo*.

The double-helical structure of DNA, in addition to suggesting a mechanism for DNA replication, also imposes a strict requirement (1). The intertwinings of the parental strands need to be continuously removed as replication proceeds. If the intertwinings between parental DNA strands are not removed, (+)-superhelical stress will prevent further unwinding of the DNA and cause replication fork movement to cease. After the final base pairs have been replicated, any remaining parental DNA intertwinings that must be removed. The enzymes that catalyze the removal of such DNA crossings, and therefore of the parental intertwinings, are DNA topoisomerases. Topoisomerases accomplish unlinking by passing one segment of DNA through a transient break in a second segment. Two types of topoisomerases, designated type-1 and type-2, have been identified (9–11). Type-1 topoisomerases catalyze the passage of DNA strands through single-strand breaks in DNA. Only the type-2 topoisomerases are capable of catalyzing strand-passage reactions between the double-stranded DNA crossings that occur in the replication intermediates described above. A typical strand-passage reaction by type-2 topoisomerases involves two distant segments of double-stranded DNA: the “gate” segment, which is transiently broken, and a segment to be passed through the gate, the “transfer” DNA segment (12, 13). The gate is opened by an ester exchange reaction in which the enzyme becomes covalently bound to the DNA via phosphotyrosyl bonds at the 5’-ends of the DNA gate (14). Binding of ATP or the non-hydrolyzable analog AMP-PNP1 induces a conformation change in the enzyme that promotes the movement of the “transfer” DNA strand through the opening in the gate strand (15, 16). ATP hydrolysis is required to regenerate the enzyme for another catalytic cycle (17–19).

The unique ability of type-2 topoisomerases to recognize and remove double-stranded DNA crossings suggests that only these enzymes can remove all the types of crossovers shown in Fig. 1. In both prokaryotes and eukaryotes, only the type-2 topoisomerases have been shown to be essential for chromosome segregation (6, 20). Interestingly, in *E. coli* and other eubacteria, two distinct type-2 topoisomerases are essential, DNA gyrase and topoisomerase IV (Topo IV) (21, 22). A recent study using mutants and inhibitors has shown that these two enzymes are responsible for unlinking DNA at different stages.

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1 The abbreviations used are: AMP-PNP, adenosine 5’-(β,γ-imido)triphosphate; Topo IV, topoisomerase IVint, λ integrase.
of replication in vivo (23). Topo IV is the primary decatenating enzyme, whereas DNA gyrase alone is sufficient for elongation synthesis. The same division of labor has been demonstrated with replicating plasmid DNA and purified enzymes in vitro (24–26).

While the introduction of (−)-supercoils into relaxed DNA is a unique ability of DNA gyrase, both DNA gyrase and Topo IV are capable of strand-passage reactions leading to decatenation and removal of (±)-supercoils (27–29). These reactions are exactly those required to unlink the crossings that exist in replication intermediates. To gain further insight into the division of labor between these enzymes in vivo, we compared the activities of DNA gyrase and Topo IV in vitro. We measured rates of DNA strand passage using supercoiled monomeric circles or catenanes that were nicked, supercoiled, or relaxed. These substrates mimic several DNA crossings predicted to occur during replication of chromosomes. The results clearly show that the two enzymes, while closely related with respect to amino acid sequence, differ strikingly in their ability to catalyze inter- versus intramolecular strand-passage reactions. Topo IV has a 10–40-fold higher specific activity for intermolecular (decatenation) reactions, while DNA gyrase favors intramolecular (supercoiling) reactions by at least 2 orders of magnitude in the reaction conditions used here. These observations extend previous results with DNA gyrase (24). The decatenating activities of both enzymes are stimulated by supercoiling. Finally, the kinetic experiments show that Topo IV can discriminate between the topological links of catenanes or knots and the plectonemic crossings of supercoils. The biochemical activities of the enzymes, therefore, closely match the demonstrated roles of each enzyme in vivo and explain the failure of either wild-type enzyme to compensate for mutations in the other.

**EXPERIMENTAL PROCEDURES**

**Materials**—The ParE subunit of Topo IV was purified according to Kato et al. (29). The ParC subunit was purified according to Kato et al. (29) up to the precipitation in low salt. After that point, the protocol of Marians and co-worker (30) was used. Purified DNA gyrase subunits A and B were the gift of Dr. David Bramhill. Bacteriophage λ Int (31), E. coli integration host factor (32), and wheat germ topoisomerase I (33) were purified as described. Bovine pancreatic DNase I (type II) was obtained from Sigma.

Plasmid pAB4 (alias pAB7.0d in Ref. 34), the substrate for Topo IV relaxation assays and DNA gyrase supercoiling assays, contains recombination sites for both Int and yh resolvase recombinases. Singly linked supercoiled catenanes consisting of one 3-kilobase and one 4-kilobase circle were generated by yh resolvase-mediated site-specific recombination of plasmid pAB4 (35). Nicked singly linked catenanes were prepared by treatment of supercoiled singly linked catenanes with DNase I in the presence of ethidium bromide. Wheat germ topoisomerase I was used to prepare relaxed singly linked catenanes. All large scale preparative reactions were stopped by heating to 70 °C followed by phenol extraction and ethanol precipitation. Singly catenated plasmids were typically 70–80% catenanes, with the remainder composed almost entirely of unreacted substrate and a small fraction of knotted and unlinked products of recombination.

Single species of multiply interlinked nicked catenanes were purified as follows. Reactions using λ Int to create multiply interlinked supercoiled catenanes of pAB4 were as described (34) except less protein was added (3.25 µg/ml Int and 2.5 µg/ml integration host factor). After reaction with Int, fully nicked DNA catenanes were generated by treatment with DNase I in the presence of ethidium bromide. Electrophoresis then extraction from low melting agarose allowed recovery of single catenate isomers (36).

Knots of molecules of nearly identical size to pAB4 were made by Int-mediated recombination of plasmids pCUH10. pCUH10 (7200 base pairs) was constructed by inserting the 2900-base pair EcoRI fragment of pBR322 (37) containing inversely repeated Int sites separated by 515 base pairs into the EcoRI site of pBR322. Preparation of supercoiled knots was according to the Int catenating protocol above. A population of approximately equal quantities of 3, 5, 7, and 9-noded supercoiled knots was generated. Knotted products constituted 70% of the final preparation, the remainder being unreacted substrate. Nicked knots of pCUH10 were prepared by treatment of supercoiled knots with DNase I in the presence of ethidium bromide.

**Methods**—Experiments measuring the rates of strand passage during relaxation, supercoiling, decatenation, and unknottyng by DNA gyrase or Topo IV were carried out under identical buffer and salt conditions (40 mM Hepes, pH 7.6, 80 mM potassium glutamate, 8 mM MgCl2, 50 µg/ml bovine serum albumin, 2 mM ATP). Reactions were performed at 23 °C. Reactions were initiated by addition of enzyme, and samples were removed at the indicated time points and stopped by addition of EDTA to 10 mM followed by proteinase K treatment. Electrophoresis was through 0.9% agarose, TAE buffer (38) for all experiments except the unlinking of multiply interlinked catenanes, which used high resolution running buffer (39). Where chlorosulfonic or ethidium bromide was added, concentrations are indicated in the figure legends. Following electrophoresis, the DNA was transferred from the gel to MSI Magna membrane followed by UV cross-linking and hybridization at 68 °C overnight with a 32P-radiolabeled, nick-translated substrate probe. DNA bands were quantitated with a Molecular Dynamics phosphorimagery.

To limit decatenation by Topo IV to a single catalytic cycle, we replaced ATP with a non-hydrolyzable analog, AMP-PNP. AMP-PNP-mediated unlinking was assayed by incubating 150 fmol of supercoiled singly linked catenanes with the indicated amount of enzyme in a buffer containing 40 mM Tris-Cl, pH 7.6, 80 mM potassium glutamate, 8 mM MgCl2, and 50 µg/ml bovine serum albumin for 10 min at 30 °C. AMP-PNP was added to a final concentration of 2 mM, and incubation was continued for 10 min. Reactions were stopped by addition of cold EDTA to a final concentration of 50 mM. After proteinase K treatment, the products of the reaction were analyzed by gel electrophoresis as above. The ratio of plectonemic supercoil crossings to catenate crossings was calculated as follows. The superhelical density of the catenanes is −0.06 (data not shown). For a 7-kilobase molecule, this corresponds to a linking deficit of 40 turns, assuming a helical repeat of 10.5 base pairs/nturn. The ratio of average writhe to ΔLk is calculated to be about 0.7 under the ionic conditions of our experiments (40). Therefore, there are approximately 30 superhelical turns per catenate crossing in each molecule. Multipled by 45%, the extent of catenane unlinking when stoichiometric amounts of Topo IV are used, this gives an estimate of 15-fold preference for catenate crossings over supercoil crossings.
Contrasting Activities of Topo IV and DNA Gyrase

The table compiles the rates obtained from experiments described in this paper. Rates are given in terms of strand-passage events min$^{-1}$/fmol enzyme. The given rate of unlinking of nicked multiply interlinked catenanes is an adjustment based on the rate of unlinking of nicked singly linked catenanes in previous experiments. The low yield of pure multiply interlinked catenanes demanded the use of significantly lower concentrations of substrate and enzyme than were used in other experiments. The measured rate of a control reaction (shown in Fig. 6) using the same low concentrations of nicked singly linked catenanes showed the rate to be 0.4 fmol linkages min$^{-1}$/f mol enzyme, five times lower than the average rate of previous experiments. The actual measured rates on the 10-noded and 20-noded catenanes were 0.2 and 0.7 linkages min$^{-1}$/f mol enzyme, respectively.

| Assay                      | Rates of strand passage |
|----------------------------|-------------------------|
|                            | Topo IV                 | DNA gyrase               |
| Supercoiling               | —a                      | 6                        |
| Relaxation                 | 0.3                     | —                        |
| Decatenaion (supercoiled   | 8                       | 8 × 10$^{-3}$            |
| catenanes)                 |                         |                          |
| Decatenaion (nicked        | 2                       | 2 × 10$^{-4}$            |
| catenanes)                 |                         |                          |
| Unknottedting (supercoiled| —                       | —                        |
| (knots)                    |                         |                          |
| Unknottedting (nicked      | —                       | —                        |
| (knots)                    |                         |                          |
| Decatenaion of multiply    | (≥2)                    | —                        |
| interlinked catenanes      |                         |                          |

a Not determined or not applicable.

The relatively low activity of relaxation versus decatenation was also suggested by the uniform gel mobility of the released supercoiled circles over the course of the decatenation reaction (Fig. 2A). Because the electrophoresis buffer in the above experiments contains saturating amounts of ethidium bromide, if the supercoiled product circles were being relaxed, their mobility would increase over time. To directly address the relaxation activity of Topo IV, we measured the rate of removal of plec tonic supercoils from uncatenated pAB4 ($\sigma = -0.06$) and found it to be 0.3 fmol strand-passage events min$^{-1}$/f mol enzyme, more than an order of magnitude slower than decatenaion of supercoiled catenanes (Fig. 3 and Table I). Also, under our conditions, unlinking of supercoiled DNA occurs distributively, with the entire population of molecules being relaxed uniformly over time. The slow rate of unlinking of plec tonic supercoils shows that Topo IV is capable of discriminating between different transfer strands on supercoiled catenanes. In this case, a reaction in which the transfer and gate strands are from two different molecules—the two rings of a catenane—is favored over the intramolecular relaxation reaction.

AMP-PNP-mediated Unlinking of Supercoiled Catenanes—

The non-hydrolyzable ATP analog AMP-PNP has proven a useful tool in dissecting the mechanism of type-2 topoisomerases because strand-passage reactions are limited to a single round (41, 42). Experiments with yeast topoisomerase II demonstrated that after addition of AMP-PNP to enzyme-bound DNA, the enzyme was inactive for strand passage but remained stably attached to the DNA via a protein-mediated topological clamp (43). Further studies showed that while the enzyme preferred to bind to the supercoiled ring of a nicked DNA catenane, addition of AMP-PNP resulted primarily in decatenation rather than relaxation of the supercoiled ring (15, 44).
The ability to look at a single round of unlinking enabled us to directly measure the probability of catenane removal in the presence of plectonemic supercoils. The data is shown in Fig. 4. At a ratio of 1:1 stoichiometric ratio, we found that 45% of the catenanes were unlinked. Even if the remaining 55% of the enzyme had relaxed a supercoil upon AMP-PNP binding, catenane crossings must be favored as substrates 15 to 1 over the juxtaposed segments of plectonemically supercoiled DNA due to the excess of the latter. This result is similar to the order of magnitude difference observed in the kinetics of crossover removal seen with catalytic amounts of enzyme.

**Unlinking by DNA Gyrase**—The other type-2 topoisomerase in *E. coli*, DNA gyrase, has a role different from Topo IV in *in vivo* in that it is important for introducing (−)-supercoils into DNA. This reaction is topologically equivalent to unlinking the strands of a DNA duplex. Only (−)-supercoils are introduced by DNA gyrase, which means that it must non-randomly select the DNA segments to be passed (9, 45, 46). It has been shown, using the multiply interlinked catenated products of *in vitro* replication reactions as substrates, that turnover numbers in terms of strand passage by DNA gyrase can be up to 130 times greater for supercoiling than for decatenation (24). Thus, DNA gyrase, like Topo IV, appears to have its distinct preferences for strand-passage reactions.

We compare the relative strand-passage activities of DNA gyrase under the identical reaction conditions used for our Topo IV kinetic experiments, using preparations of singly linked catenanes that are nicked or negatively supercoiled as substrates. Data from a typical decatenation reaction are shown in Fig. 5. Note that 5-fold more DNA gyrase was used in the experiment to measure the unlinking of nicked catenanes. The calculated turnover rate of DNA gyrase in units of strand passages min⁻¹ is shown in Table I. There are several important results. The first is that, like Topo IV, the unlinking of catenanes is stimulated by supercoiling. In the case of DNA gyrase, unlinking of supercoiled catenanes is 40 times faster than the unlinking of nicked catenanes. The lower rate of decatenation of nicked rings is due to their topology, not simply a direct inhibitory effect of the nick. Relaxed unnicked DNA catenanes are supercoiled and decatenated by DNA gyrase nearly as quickly as catenanes that were supercoiled before the addition of enzyme (data not shown). That DNA gyrase first supercoils and then decatenates the rings was supported by the following experiment. When supercoiling by DNA gyrase is counteracted by the addition of sufficient wheat germ Topo I, the rate of decatenation of relaxed catenanes approaches that of nicked catenanes (<0.0008 strand passages min⁻¹/fmol DNA gyrase).

The second key result is that the rate of decatenation by DNA gyrase of nicked or supercoiled singly linked catenanes is 3 or 4 orders of magnitude less than the rate of supercoiling by DNA gyrase—

**FIG. 4.** AMP-PNP-mediated unlinking of supercoiled catenanes by Topo IV. AMP-PNP instead of ATP was added after a 10-min preincubation of catenanes with Topo IV (see “Experimental Procedures”). 150 fmol of singly linked supercoiled DNA catenanes were used in each reaction. The ratios above each lane refer to the molar ratios of enzyme to DNA catenanes. − and + symbols refer to the absence or presence of AMP-PNP. Electrophoresis was through 0.9% agarose-TAE in the presence of 2 μM ethidium bromide.

**Fig. 3.** Removal of plectonemic supercoils by Topo IV. Supercoiled pAB4 (175 fmol) was incubated with 20 fmol of Topo IV. Samples were removed at the indicated times, the reaction was stopped, and DNA topoisomers were resolved by electrophoresis through a 0.8% agarose gel in TAE buffer plus 1.5 μg/ml chloroquine.

**Fig. 5.** Unlinking of supercoiled and nicked catenanes by DNA gyrase. A, 125 fmol of supercoiled or nicked catenanes were incubated with DNA gyrase. 40 and 200 fmol of DNA gyrase was used to unlink supercoiled catenanes and nicked catenanes, respectively. Samples were taken from reactions at the indicated times. Bands migrating above supercoiled catenanes are nicked and linear forms of catenanes and substrate DNA. B, quantitation of the data in A.
Contrasting Activities of Topo IV and DNA Gyrase

The ability of Topo IV to discriminate between catenane crossings and plectonemic supercoil crossings was unexpected. How does the enzyme tell the difference between intramolecular and intermolecular passage strands? One explanation for the difference in rates of unlinking of plectonemic supercoils and catenane crossings by Topo IV is that the enzyme is sensitive to the local geometry of the segments to be transposed. Monte Carlo simulations of the conformation of circular DNA show that the mean angle of juxtaposition of DNA segments decreases from 90° when the DNA is relaxed to 60° after supercoiling (47). The angle of juxtaposition of catenane crossings in relaxed singly linked catenanes is predicted to have its center of distribution at 90° (47). Supercoiling of the individual rings should not change the mean angle of the catenane crossing. An intrinsic bias of Topo IV for a 90° angle also seems reasonable because passing strands at such an angle would minimize the width that the DNA gate must open to allow the second DNA duplex to pass through.

Multiply linked catenanes were used to test the angle model. Increasing catenane complexity (i.e. multiple interlinking) induces writhe in the component rings (48) and would be accompanied by a decrease in the angle of juxtaposition of catenane crossings. If Topo IV is sensitive to acute angles of segment juxtaposition, then multiple interlinking of catenanes should inhibit the rate of strand passage. This assumes that there is not a compensatory increase in the frequency of site juxtaposition because of the reduction in the mean distance between the two rings of the catenanes (49). Single species of nicked catenanes with five or ten interlinks (10- or 20-noded) were isolated, and turnover numbers were determined. Fig. 6 shows an unlinking reaction, which is quantified in Table I. The data show that, rather than retarding decatenation rates, multiple interlinking stimulates the reaction rate somewhat compared to the unlinking of singly linked catenanes. Therefore, the acute angles of segment juxtaposition in multiply interlinked catenanes do not affect the measured rate of decatenation by Topo IV, at least at the extent of catenation used here.

A second possible model for the discriminatory activity of Topo IV is that intramolecular crossovers are intrinsically more difficult to remove, perhaps because the slithering of two intramolecular segments prevents their binding stably to the enzyme (50, 51). Knotted DNAs provide a means of testing this model. The crossings of DNA segments in knots are intramolecular, like the plectonemic crossings in supercoiled DNA. Supercoiled or nicked DNA knots were used as substrates in Topo IV unlinking reactions. The data shown in Fig. 7 and summarized in Table I demonstrate that Topo IV unknots and decatenates DNA at similar rates. Therefore, supercoil crossovers are not resistant to unlinking by Topo IV simply by virtue of being intramolecular. Also, supercoiling of knots, in contrast to the effect observed with catenanes, did not appreciably increase the rate of their unlinking by Topo IV (Fig. 7, Table I). These results are discussed below.

DISCUSSION

In this study, we measured the specific activities of Topo IV and DNA gyrase on substrates of differing topology. The most potent activities—the introduction of (∼)-supercoils into a relaxed DNA circle by DNA gyrase and the unlinking of supercoiled catenanes by Topo IV—correspond to the major roles of the enzymes in vivo and are roughly equal in terms of rates of strand passage (Table I). This shows that the catalytic efficiency of these homologous enzymes is about the same, and the differences between strand-passage activities on various substrates are inherent to the enzyme’s mechanisms. Topo IV decatenates supercoiled DNA (whether singly linked or multiply linked) 10 to 40 times more readily than it catalyzes strand-passage events leading to relaxation of DNA. DNA gyrase, on the other hand, introduces supercoils into DNA 10^3 times more quickly than it decatenates. Thus, the ratio of rates of inter- to intramolecular passage events is 10^4 times greater for Topo IV than DNA gyrase. Supercoiling of catenanes actually stimulates unlinking by Topo IV as well as DNA gyrase. Unlinking of knots by Topo IV is not stimulated by supercoiling but is accomplished as readily as decatenation.

These results are consistent with observations made by other
laboratories. The reported $K_m$ with respect to ATP concentration is at least an order of magnitude lower for decatenation by Topo IV than for relaxation (30), suggesting that ATP is more efficiently coupled to strand passage during decatenation than during relaxation of supercoils. Similarly, when ATP is not limiting, relaxation by *Drosophila* or yeast topoisomerase II consumes severalfold more ATP per strand-passage event than is required (52, 53). Roca *et al.* have observed that yeast topoisomerase II exhibits similar properties to Topo IV, favoring decatenation of supercoiled molecules (13, 44) as well as knotting over relaxation (54).

The relatively weak decatenation activity of DNA gyrase can be understood as the inevitable consequence of its remarkable supercoiling activity. To introduce negative supercoils into DNA, a vectorial reaction, DNA gyrase must choose the segments to be cleaved and transferred non-randomly. DNA gyrase accomplishes this by wrapping about itself a long (135 base pairs) stretch of DNA, presumably including both the gate and transfer segments, in a right-handed supercoil (55, 56). If the formation of such a complex is energetically favorable (i.e., the substrate DNA is relaxed or positively supercoiled), the binding of a non-contiguous or intermolecular transfer strand will be disfavored, and reactions leading to supercoiling will dominate (57). When DNA is negatively supercoiled, however, the right-handed wrapping of DNA around DNA gyrase is unfavorable. In this case, binding of a non-contiguous passage strand becomes more probable and could account for the increased rate of decatenation of supercoiled catenanes observed experimentally (Ref. 24 and this study). The stimulation of DNA gyrase's decatenation activity in solutions of low ionic strength may similarly result from an impaired ability to form the competing DNA-protein complex that leads to supercoiling (24).

The stimulatory effect of supercoiling on decatenation by Topo IV was unexpected. We anticipated that the plectonemic crossings of DNA segments in supercoiled molecules would compete with the catenane crossings for Topo IV binding and thus retard catenane unlinking. A possible explanation for stimulation by supercoiling is that Topo IV acts preferentially on apical loops of supercoiled DNA where catenane nodes may be localized (58). Evidence to support the location of type-2 topoisomerases at apical loops is lacking, however. A second possibility is that the demonstrated preferential binding of Topo IV to supercoiled DNA could stimulate decatenation of supercoiled catenanes (59). This would only be true if DNA binding were the rate-limiting step in strand passage. The fact that the plectonemic crossings of supercoiled DNA circles are themselves removed relatively slowly suggests that this explanation is insufficient. A third possibility is that the increase in decatenation rates could result from an increase in juxtaposition of catenane crossings as supercoiling decreases the volume occupied by the two linked circles. Theoretical and experimental data show that the equilibrium probability of catenation decreases several orders of magnitude when DNA rings become supercoiled (47). This possibility could account for some of the stimulation of decatenation by supercoiling observed both with Topo IV and DNA gyrase.

Yeast topoisomerase II behaves virtually identically to Topo IV in its preferential removal of intermolecular linkages from supercoiled catenanes (44). The simplest explanation for the preferential unlinking of catenane crossings by these enzymes is that strand passage is facilitated when the strand to be transferred intersects with the gate segment of DNA at a 90° angle. This angle makes intuitive sense given the symmetrical structure of type-2 topoisomerases (44, 60) and the fact that strand passage at a perpendicular angle minimizes the gaping required at the cleaved strand. Vologodskii and co-worker (47) have calculated that the probability of 60° angle juxtapositions of distant segments in supercoiled circles is approximately 5 times that of 90° angle juxtapositions. The recognition by Topo IV of crossover geometry at several steps during the strand-passage reaction could account for the greater degree of discrimination observed relative to the calculated free energy differences. For instance, a perpendicular approach by the transfer strand may provide the correct steric repulsion for optimal separation of the gate strand (60).

Testing this model is severely complicated by an absence of substrates in which the predicted angle of segment juxtaposition can be changed without affecting the concentration of juxtaposed segments. The experiments in this paper that show facile unlinking of multiply interlinked nicked catenanes and supercoiled knots by Topo IV do not support the model. They do show that type-2 topoisomerases are not simply cleavage ligases with no ability to discriminate between various types of DNA crossings. In fact, when closed circular DNA is treated with catalytic amounts of Topo IV, the observed distribution of relaxed DNA topoisomers is narrower than that achieved with eukaryotic type-1 topoisomerases or ligation of nicked circles (data not shown). Similarly, the observed level of catenation of DNA circles in solution with type-2 topoisomerases is sharply decreased relative to the chemical equilibrium achieved in the absence of enzyme. Taken together with the results of this paper, these observations imply that proposed mechanisms for type-2 topoisomerases in which only two DNA segments are recognized may be incorrect.

While we are not yet certain of the physical basis for the differences between Topo IV and DNA gyrase, the physiological importance is clear. These two closely related enzymes have evolved to contribute very differently to the unlinking of DNA. The essential role of Topo IV as a decatenase has become clear. Experiments *in vitro* show that Topo IV is necessary for resolution of catenanes following replication (25, 26, 30) as well as for efficient processing of late replication intermediates in some systems (61). *In vivo*, Topo IV is responsible for the unlinking of supercoiled catenanes resulting from replication of plasmid DNA (23).

The evolution of type-2 topoisomerases in *E. coli* may have resulted from two selective forces. The need to decatenate replicated chromosomes efficiently demands an enzyme like Topo IV. An additional demand for rapid DNA replication may have led to the evolution of a powerful (+)-supercoil-removing enzyme, DNA gyrase. Eukaryotes have the essential decatenating activity but lack an enzyme like DNA gyrase. In prokaryotes, the introduction of (−)-supercoils by DNA gyrase has the additional effect of compacting the genome (62, 63). Supercoiling of replicated chromosomes could compact the chromosomes and facilitate their unlinking by Topo IV (47). The failure of DNA gyrase to supercoil chromosomes in some *gyrB* mutants (64) may lead to reduced chromosome decatenation by Topo IV. This indirect effect of DNA gyrase may be the cause of the dumbbell nucleoids in these conditionally lethal strains.

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