Two Distinct Modes of Strand Unlinking during \( \theta \)-Type DNA Replication*

(Received for publication, December 6, 1995, and in revised form, June 7, 1996)

Hiroshi Hiasa and Kenneth J. Marians

From the Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

DNA gyrase and topoisomerase IV (Topo IV) are type II bacterial DNA topoisomerases that show a high degree of similarity to each other yet appear to have different functions during DNA replication. We show here that the torsional stress generated during \( \theta \)-type DNA replication in vitro can take the form of either positive supercoils ahead of the replication fork or catenane-like right-handed windings (precatenanes) of the two partially replicated duplexes behind the fork. Gyrase prefers to act on the former, whereas Topo IV prefers the latter. Removal of either form of positive winding can support nascent chain elongation, but only precatenane removal can support the final stages of DNA replication: processing of the late intermediate and daughter chromosome decatenation.

The requirement for topoisomerase action during DNA replication is observed in all organisms. Most prokaryotic chromosomes are circular, whereas linear eukaryotic chromosomes are anchored to the nuclear matrix. Thus, in each case, free rotation of the strands of DNA about each other is prevented, creating a topological domain. Because any permanent alteration of the linking number of the two DNA strands within a topologically constrained domain is forbidden, the unwinding of the parental duplex DNA during replication generates excess positive windings. If the topological domain is negatively supercoiled, some of these excess positive windings can be annihilated; however, eventually their accumulation will cause DNA replication to cease. Topoisomerases act to remove these excess positive windings and are therefore required for DNA replication in both prokaryotes and eukaryotes (1).

During the terminal stages of DNA replication, topoisomerase action is required for processing of the late replicative intermediate (LRI)\(^1\) (6, 7), where the two daughter duplexes are joined by about 200 base pairs of unreplicated parental DNA, and for decatenation of the linked daughter chromosomes (7, 8).

We have used the replication of oriC plasmid DNAs reconstituted with purified proteins as a model system to study the action of the topoisomerases. We have shown that of the four Escherichia coli topoisomerases, three (DNA gyrase, topoisomerase III (Topo III), and topoisomerase IV (Topo IV)) are able to support nascent chain elongation (9, 10), whereas only two (Topo III and Topo IV) are capable of supporting processing of the LRI and therefore subsequent chromosome decatenation (6, 7). Although gyrase can decatenate multiply linked DNA dimers (11), it fails to do so during oriC DNA replication because it cannot support processing of the LRI (6). Topoisomerase I cannot support any stage of DNA replication (6, 10).

Topo IV and gyrase are both ATP-dependent type II topoisomerases (12–16), although only gyrase can supercoil DNA. These enzymes share 36–40% amino acid sequence homology (17), yet they display different properties during DNA replication (6, 7, 9). In order to understand the basis for these differences, we have analyzed the action of gyrase and Topo IV on the two types of positive windings that arise during DNA replication: positive supercoils and right-handed catenanes.

Gyrase bound both positively supercoiled DNA and multiply linked DNA dimers with roughly equal affinity, whereas Topo IV displayed a 5-fold higher affinity for the latter form. On the other hand, gyrase was 4-fold more active on positively supercoiled DNA, whereas Topo IV was 30-fold more active on multiply linked DNA dimers. This suggested that these enzymes might act differently during \( \theta \)-type DNA replication, with gyrase and Topo IV removing excess positive windings present as positive supercoils and catenane-like windings of the two partially replicated duplexes (precatenanes (18)), respectively. Positive supercoil removal for both enzymes was salt-sensitive, whereas catenane unlinking was salt-sensitive. We used this differential salt sensitivity during the oriC replication reaction in an attempt to differentiate the contributions of these two modes of strand unlinking in supporting DNA replication.

Elongation of nascent chains was insensitive to salt, whereas LRI processing was sensitive, suggesting that either mode of strand unlinking could support the former but that precatenane unlinking was required for the latter. Precatenane removal alone was clearly sufficient to support elongation because Topo III, which cannot relax positive supercoils, could support elongation. Moreover, we showed that the distribution of excess positive windings as precatenanes or positive supercoils was a function of the extent of replication, because the rate of Topo III-supported strand elongation increased and that of gyrase-supported chain elongation decreased as the extent of template that was replicated increased.

**MATERIALS AND METHODS**

Replication Proteins and Plasmid DNAs—E. coli DNA replication proteins and topoisomerases were as described previously (5, 15, 19, 20). Two types of oriC plasmids, pBROTB535 type I (21) and pBROTB353 type I (22), were prepared according to Marians et al. (23).

oriC DNA Replication—The standard oriC DNA replication reaction was as described previously (21). DNA topoisomerases were added to the reaction mixture as indicated in the figure legends. Gel electrophoretic analyses of replication products were performed according to Hiasa and Marians (21).
Topoisomerase Action during DNA Replication

Pulse-chase Analysis of Replication Products—Pulse-chase analysis for orIC DNA replication was as described previously (6). Changes in conditions are indicated in the figure legends. DNA products were analyzed by electrophoresis through neutral agarose gels. Gels were dried and exposed to x-ray film. Quantitation of replicative intermediates was performed using a Fuji BAS1000 phosphorimager. Pulse-chase analysis to follow the maturation of preformed ERI was as described by Hiasa and Marians (9). Changes in conditions are indicated in the figure legends. DNA products were analyzed by electrophoresis through neutral and alkaline agarose gels, and the replicative intermediates were quantitated as described above.

Preparation of Positively Supercoiled DNA—Form II DNA molecules were purified from orIC replication reaction mixtures containing DNA gyrase and Topo IV but not DNA ligase. Reaction mixtures (200 μl) contained 40 mM Hepes-KOH (pH 8.0), 10 mM magnesium acetate, 10 mM dithiothreitol, 100 μg/ml bovine serum albumin, 50 μM NAD, 40 μM each of dATP, dGTP, dCTP, and TTP, 4 μg/ml tRNA, and 32P-form II DNA molecules prepared from orIC DNA replication reactions containing 350 fmol of template DNA. DNA gyrase (2.5 pmol) was bound to the DNA in an incubation of 15 min at 30°C. DNA ligase (14 nm) and DNA polymerase I (19 nm) were then added, and the incubation continued for an additional 45 min at 5°C. The reaction was stopped by adding EDTA to 20 mM followed by the addition of NaCl, SDS, and proteinase K, to 200 mM, 0.1%, and 5 μg/ml, respectively, followed by another incubation of 30 min at 30°C. The reaction mixture was deproteinized by phenol extraction, and the DNA was recovered by ethanol precipitation. DNA products were analyzed by electrophoresis through native agarose gels in the presence and absence of 13 μg/ml chloroquine phosphate to confirm the presence of positive supercoils. The gels were dried and exposed to phosphorimager screens and then to x-ray film.

Relaxation of Positively Supercoiled DNA—Standard reaction mixtures (12.5 μl) contained 40 mM Hepes-KOH (pH 8.0), 10 mM magnesium acetate, 10 mM dithiothreitol, 100 μg/ml bovine serum albumin, 2 mM ATP, 4 μg/ml tRNA, and positively supercoiled DNA (equivalent to 35 fmol of DNA template from the original replication reaction mixture). 400 mM potassium glutamate was added as indicated. Topoisomerases were added as indicated, and the reaction mixtures were incubated at 30°C for the indicated times. Reactions were stopped by the addition of EDTA to 25 mM, and the DNA was analyzed as described in the previous section.

Decatenation of Multiply Linked DNA Dimers—Multiply linked DNA dimers were purified as described by Marians (11) by sucrose gradient centrifugation from the orIC replication reaction (in the absence of Topo IV) increased in size by 100-fold. Decatenation reaction mixtures (5 μl) containing 50 μM Hepes-KOH (pH 8.0), 6 mM MgCl2, 5 mM dithiothreitol, bovine serum albumin at 100 μg/ml, DNA dimers (4 fmol as monomer), and the indicated amounts of topoisomerase were incubated at 30°C for 30 min. The reactions were stopped by the addition of EDTA to 20 mM. Gel loading dye was then added, and the reactions were analyzed by electrophoresis through vertical 0.8% agarose gels at 2 V/cm for 18 h. The gels were dried, exposed to phosphorimager screens, and autoradiographed.

DNA Binding Assay—DNA binding reaction mixtures (10 μl) containing 2 μM (as monomer) DNA substrate, 50 mM Tris-HCl (pH 7.6 at 30°C), 20 mM KCl, 10 mM MgCl2, 2 mM dithiothreitol, 50 μg/ml bovine serum albumin, and the indicated amounts of either gyrase or Topo IV were incubated at 30°C for 20 min. Washing buffer (0.2 ml of 25 mM Tris-HCl, pH 7.5, 20 mM KCl, 5 mM MgCl2, 1 mM EDTA, and 10 mM β-mercaptoethanol) was then added, and the reactions were passed through nitrocellulose filters (Millipore Corp.) at 1 ml/min. The filters were then washed three times with 1 ml of washing buffer. The filters were dried, and the radioactivity retained was determined by liquid scintillation spectrometry.

RESULTS

Differential Activity of Topo IV and Gyrase on Positive Supercoils and Right-handed Catenanes—Two related types of positive windings arise during θ-type DNA replication: positive supercoils and right-handed catenane linkages between the two daughter chromosomes. In order to better understand the biochemical basis for the observed difference in action between Topo IV and gyrase during DNA replication, we assessed the catalytic and DNA binding activities of these enzymes, using as a substrate either positively supercoiled DNA or multiply linked DNA dimers.

Positively supercoiled DNA uniformly labeled with 32P was prepared by binding DNA gyrase in the absence of ATP to form II DNA that had been isolated from orIC DNA replication reactions. Because gyrase wraps about 150 bp of DNA about itself in a positive toroidal supercoil when bound (24), sealing of the form II DNA via the action of DNA polymerase I and DNA ligase followed by deproteinization results in the production of covalently closed duplex circular DNA containing interwound positive supercoils. The DNA preparations had an average of six or seven positive supercoils (Fig. 1). 32P-multiply linked DNA dimers were purified from orIC replication reactions where gyrase was the only topoisomerase present as described previously (7). These DNA preparations consisted of two form II DNA molecules linked together on average 30 times (LKi = 30, (11)) (Fig. 2).

Enzyme titrations were used to determine a relative kcat for Topo IV and gyrase when either positive supercoils (Fig. 1) or multiply linked DNA dimers (Fig. 2) were used as the substrate. The time of incubation in these experiments gave initial reaction rates in all cases as determined by kinetic analysis (data not shown). Topo IV relaxed the positive supercoils, generating form I DNA via the action of DNA polymerase I and DNA ligase (electrophoretically indistinguishable from form II on this gel), whereas gyrase converted them directly into negative supercoils, generating form I DNA (Fig. 1A). The reaction rates were determined by quantitation using a phos-
phorimager and are presented in Fig. 1. Bas strand passage events/min as a function of topoisomerase concentration. Gyrase showed an average apparent $k_{cat}$ of 0.27/min over the range of concentrations tested, whereas Topo IV activity was roughly the same, 0.16/min, at low concentrations. The rate saturated at higher concentrations. Interestingly, Topo IV appeared to act distributively in this reaction (all of the positively supercoiled DNA was relaxed at the same time), whereas gyrase appeared to act processively (fully supercoiled form I product could be observed when there was still positively supercoiled DNA remaining), as it does during the generation of negative supercoils (12).

It should be noted that because the preparation of positively supercoiled DNA contained some form II DNA (about 25% of the total), the actual $k_{cat}$ values are likely to be higher than the apparent values given above. The magnitude of the difference is difficult to estimate because these are large DNAs containing multiple topoisomerase binding sites that differ in affinity for the enzymes.

The ability of the two topoisomerases to unlink catenated DNA differed dramatically (Fig. 2). The average $k_{cat}$ before rate saturation was 5.0 strand passage events/min for Topo IV, but only 0.07 for gyrase, nearly a 70-fold difference (Fig. 2B). In addition, the nature of the reaction mechanism was reversed compared with that observed with positively supercoiled DNA. Here Topo IV appeared to act processively (the final decat-
cation fork as positive supercoils (27). Champoux (28) pointed out some time ago that nothing prevents the excess positive windings from equilibrating across the replicating template to take the form of positive windings of the two partially replicated daughter duplexes about each other in a catenane; therefore, they have named this form of excess positive windings in a replicating template a precatenane.

We therefore considered the possibility that the difference between Topo IV and gyrase activity during \( \theta \)-type DNA replication resulted from a requirement for precatenane removal during one stage of the replication process. Previous studies had shown that both Topo IV (7) and gyrase-catalyzed (11) decatenation of multiply linked DNA dimers was sensitive to salt. On the other hand, we found (Fig. 5) that both gyrase-catalyzed conversion of positive supercoils to negative ones and Topo IV-catalyzed relaxation of positive supercoils were insensitive to 400 mM potassium glutamate. We used this differential salt sensitivity of precatenane removal, as opposed to positive supercoil removal, to dissect the mechanisms of topological processing during \( \theta \)-type DNA replication.

Only Precatenane Unlinking Supports the Terminal Stages of \( \theta \)-Type DNA Replication—We have used oriC DNA replication reconstituted in the presence of DnaA, DnaB, DnaC, DnaG, the single-stranded DNA-binding protein, the HU protein, the DNA polymerase III holoenzyme, and a pBR322-based plasmid DNA carrying oriC as template (21) to study the action of the topoisomerases during DNA replication. We have developed a pulse-chase analysis that allows separate observation of the elongation and LRI processing stages of \( \theta \)-type DNA replication (9). Because initiation of replication at oriC is inhibited by salt (6), the pulse-chase protocol was modified so that the salt sensitivity of the elongation and terminal stages of replication could be observed separately. Reaction mixtures were assembled in the absence of topoisomerases and pulsed with [\( \alpha ^{32} \)P]dATP. Because the DNA template is supercoiled, initiation occurs, but because no topoisomerase is present to support elongation, DNA products accumulate as early replication intermediates (ERIs) containing nascent leading strands of about 600 nt (9). Topoisomerases and 400 mM potassium glutamate, as indicated, were added at the same time as the cold chase of the label, and the reaction followed kinetically. Neutral agarose gels were used to analyze formation and process- ing of the LRI (Fig. 6), and denaturing alkaline agarose gels were used to observe elongation of the nascent chains (Fig. 7).

Even in the presence of saturating levels of both gyrase and Topo IV (each at a topoisomerase:DNA ratio of 3:1), the elongation and terminal stages of DNA replication could be distinguished by their differential sensitivity to salt. In the absence of salt, processing of the LRI and decatenation leading to form II production (these reactions did not contain DNA ligase) was very rapid, essentially being complete within 2 min postchase (Fig. 6, A and D). In the presence of potassium glutamate, replication proceeded to the LRI stage, indicating that elongation was unaffected, but the rate of subsequent processing of the LRI and formation of form II production decreased by a factor of 8 (Fig. 6, A and D). Similar results were observed when either Topo IV (Fig. 6B) or gyrase (Fig. 6C) was used as the only topoisomerase present in the reaction mixture.

The rate of elongation of nascent chains was examined by measuring the length of the longest elongated product in the pulse-chase reaction 10 s after the chase (Fig. 7). This length, divided by the time, represents a direct measurement of the rate of chain elongation. In all cases examined, this rate was unaffected by the presence of potassium glutamate.

Thus, elongation, but not the terminal stages of replication, could proceed in the presence of salt. This indicates that either precatenane or positive supercoil removal could support elongation but that only precatenane unlinking could support the terminal stages of replication.

Precatenane Unlinking Can Support Nascent Chain Elongation—The results in the previous section indicated that precatenane unlinking was required for LRI processing and that either precatenane unlinking or positive supercoil removal could support nascent chain elongation, but the results could not be used to distinguish whether precatenane unlinking alone could support chain elongation. To address this question, we considered the activity of Topo III in the oriC replication system.

Topo III is a type I topoisomerase that requires a single-stranded DNA binding site for activity (29). Our previous studies had established that Topo III could support both elongation of nascent chains as well as LRI processing and subsequent daughter chromosome decatenation (10). As shown in Fig. 5, Topo III could not relax positively supercoiled DNA. This is consistent with the observation that the enzyme binds only single-stranded DNA (29) and that the yeast Topo III relaxes positively supercoiled DNA only if the DNA contains a single-stranded loop (30). Thus, Topo III must be supporting \( \theta \)-type DNA replication.
As long as equilibration of positive windings across the junction between the unreplicated duplex template and the partially replicated DNA is unimpeded by the replication fork machinery, the distribution of the excess positive windings as either precatenanes or positive supercoils should depend on the extent of the template replicated (18). At early times, positive supercoils should predominate because the majority of the template is unreplicated. If this is the case, then one would expect that the rate of Topo III-supported chain elongation should exhibit an initial lag, because the majority of excess positive windings will exist as positive supercoils in the unreplicated region of the template and then increase with time as more of the template is replicated, allowing the equilibrium to shift to favor the existence of precatenanes. This proved to be the case (Fig. 8).

The kinetics of Topo III- and gyrase-supported nascent chain elongation from preformed ERIs was determined by observing the length of the longest leading strands present on alkaline-agarose gels at time points taken at 5-s intervals postchase (Fig. 8). The rate of gyrase-supported chain elongation remained relatively constant over the first 10 s (240 nt/s, 0–5 s; 270 nt/s, 5–10 s) but then decreased by more than 50% during the next 5 s (110 nt/s, 10–15 s) (Fig. 8B). On the other hand, Topo III-supported chain elongation exhibited a dramatic lag, with no appreciable nascent chain growth during the first 5 s, followed by an acceleration of the rate in each of the next two 5-s intervals (240 nt/s, 5–10 s; 490 nt/s, 10–15 s).

It seems very likely that the observed lag and then increas-
ing rate of chain elongation observed in the Topo III-supported reaction arises from a paucity of precatenanes in the ERI and their increasing accumulation as the template is replicated. Similarly, because the bulk of the excess positive windings in the ERI exists as positive supercoils, gyrase-supported chain elongation commences immediately but then slows down as the template becomes replicated and as a greater fraction of the positive windings take the form of precatenanes.

**DISCUSSION**

In an effort to understand the biochemical basis for their differential action, we have examined the activity of the two type II DNA topoisomerases found in *E. coli*, DNA gyrase and Topo IV, using as a substrate the two types of positive windings that arise during θ-type DNA replication, interwound positive supercoils and right-handed catenane-like windings of two duplexes about each other.

Topo IV exhibited a marked preference for catenane linkages over positive supercoils in both equilibrium binding (5-fold) and turnover number (30-fold). The preference of gyrase was the opposite, although not nearly as extreme: equilibrium binding to the two forms was roughly equal, and the turnover number with a positively supercoiled substrate was about 4-fold greater than with the multiply linked DNA dimers.

In direct comparison, Topo IV was by far the better decatenating enzyme, with a $k_{cat}$ nearly 70-fold greater than that of gyrase. In addition, in this reaction Topo IV action was processive, whereas gyrase action was distributive. On the other hand, Topo IV-catalyzed relaxation of positive supercoils and gyrase-catalyzed conversion of positive to negative supercoils proceeded with roughly equivalent $k_{cat}$ values. In this reaction, however, it was gyrase that acted processively and Topo IV that acted distributively.

These observations suggested that the enzymes might act differently during DNA replication, with Topo IV preferring to unlink precatenanes accumulating behind the fork and gyrase preferring to remove positive supercoils ahead of the fork. We took advantage of the fact that decatenation of multiply linked DNA dimers and thus, presumably, precatenane unlinking by both enzymes was inhibited by salt, whereas positive supercoil removal was salt-insensitive to demonstrate that both modes of strand unlinking existed during θ-type DNA replication. The accumulation of LRI during oriC replication at high concentrations of potassium glutamate indicated that precatenane unlinking was in fact required in order for the final stages of DNA replication to proceed. This is consistent with the idea that the distribution of excess positive windings during DNA replication as either precatenanes or positive supercoils was a function of the extent of the template replicated. At the very late stages of θ-type DNA replication, when there is only 100–200 bp of unreplicated DNA remaining, all excess positive windings that arise as a result of replication fork progression will exist primarily as precatenanes.

These data present a fundamentally different picture of the fate of the positive windings generated by the unwinding of the parental template during DNA replication. Instead of accumulating exclusively ahead of the advancing replication fork as often pictured in textbooks (e.g. Kornberg and Baker (31)), the positive windings must equilibrate across the template, taking the form of precatenanes behind the forks and positive supercoils ahead of them. Equilibration is apparently unhindered by the huge replicative protein complexes at the fork.

The equilibration of precatenanes and positive supercoils could also be observed when Topo III-supported DNA replication was compared with the gyrase-supported reaction. Topo III was shown to be incapable of relaxing positive supercoils, thereby proving that precatenane unlinking could support nascent chain elongation as well as LRI processing. In addition, Topo III-supported chain elongation from the ERI showed a distinct lag and then increased in speed, whereas gyrase-supported chain elongation commenced immediately and then slowed down. This is consistent with the excess positive windings existing primarily as positive supercoils and precatenanes.
at early and late times, respectively, during replication.

The data described here also raise an interesting question. The measured rates of nascent chain elongation supported by Topo IV and gyrase are about 250 nt/s. Thus, on a bidirectionally replicating DNA, 50 excess positive windings are generated per second. Both Topo IV and gyrase saturate the rate of DNA synthesis at a ratio of three enzyme molecules per input template in the oriC replication reaction. Using the appropriate $K_D$ to determine an effective active concentration of enzyme and the $k_{cat}$ values obtained for the various reactions, we can calculate a rough relative unloading rate for both gyrase-catalyzed positive supercoil removal and precatenane unloading, as well as Topo IV-catalyzed positive supercoil relaxation of $2 \times 10^{-3}$ linkages/s and for Topo IV-catalyzed precatenate removal of 0.13 linkages/s. Neither value comes near the presumed required unloading rate.

It is certainly possible that the rate of unloading during DNA replication is increased by virtue of an interaction between the topoisomerase and the replication machinery. However, Zechiedrich and Cozzarelli (32) have determined the combined decatenation rate in vivo of Topo IV and gyrase to be $1/s$, similar to the values derived here. In addition, these authors found the relative rate of decatenation to be 100:1 in favor of Topo IV, also similar to the 70-fold difference noted here. Thus, we must consider that the majority of unloading actually occurs after replication is completed.

ERI accumulates as a stable intermediate with paused replication forks that are released as soon as a topoisomerase is added or if the topological constraint is removed by linearizing the template. Thus, the presence of positive supercoils ahead of the fork, the predominant form that would be taken by the template in the DNA synthesis at a ratio of three enzyme molecules per input replication fork and Topo IV is behind it, it is a collision between the fork and a frozen gyrase-quinolone-DNA complex that proves to be a lethal event, preventing completion of replication and possibly generating a double-strand break in the chromosome. Collisions of similar consequence between a fork and a Topo IV-quinolone-DNA complex should be far less frequent. Khodursky et al. (34) have reached identical conclusions based on their genetic studies.

Acknowledgment—We thank Dr. N. Cozzarelli for comments on these studies.

REFERENCES

1. Wang, J. C. (1985) Annu. Rev. Biochem. 54, 665–697
2. Kreuzer, K. N., and Cozzarelli, N. R. (1979) J. Bacteriol. 140, 424–435
3. Filutowicz, M., and Jonczyk, P. (1983) Mol. & Gen. Genet. 191, 282–287
4. Kaganji, J. M., and Kornberg, A. (1984) Cell 36, 183–190
5. Minden, J. S., and Marians, K. J. (1985) J. Biol. Chem. 260, 9316–9325
6. Hiasa, H., DiGate, R. J., and Marians, K. J. (1994) J. Biol. Chem. 269, 2093–2099
7. Peng, H., and Marians, K. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8571–8575
8. Adams, D. E., Shekhtman, E. M., Zechiedrich, E., Schmid, M. B., and Cozzarelli, N. R. (1992) Cell 71, 277–288
9. Hiasa, H., and Marians, K. J. (1994) J. Biol. Chem. 269, 16371–16375
10. Hiasa, H., and Marians, K. J. (1994) J. Biol. Chem. 269, 32653–32659
11. Marians, K. J. (1987) J. Biol. Chem. 262, 10362–10368
12. Morrison, A., Higgins, N. P., and Cozzarelli, N. R. (1980) J. Biol. Chem. 255, 2211–2219
13. Mizuuchi, K., Fisher, L. M., O’Dea, M. H., and Gellert, M. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1847–1851
14. Kato, J. Suzuki, H., and Ikeda, H. (1992) J. Biol. Chem. 267, 25676–25684
15. Peng, H., and Marians, K. J. (1993) J. Biol. Chem. 268, 24481–24490
16. Peng, H. (1995) Molecular Cloning, Purification, and Characterization of Escherichia coli Topoisomerase IV. PhD. thesis, Cornell University
17. Kato, J. Nishimura, Y., Imamura, R., Niki, H., Hiraga, S., and Suzuki, H. (1990) Cell 63, 393–404
18. Ullisgerger, C., Volodkovich, A., and Cozzarelli, N. R. (1995) in Nucleic Acids and Molecular Biology, Vol. 9 (Lilly, D., and Ekstedt, F., eds) pp. 115–142, Springer-Verlag, Berlin
19. Parada, C. A., and Marians, K. J. (1991) J. Biol. Chem. 266, 18895–18906
20. Wu, C. A., Zechiner, E. L., and Marians, K. J. (1992) J. Biol. Chem. 267, 4030–4044
21. Hiasa, H., and Marians, K. J. (1994) J. Biol. Chem. 269, 6058–6063
22. Hiasa, H., and Marians, K. J. (1994) J. Biol. Chem. 269, 26859–26968
23. Marians, K. J., Soeller, W., and Zipursky, S. L. (1982) J. Biol. Chem. 257, 5656–5662
24. Klevan, L., and Wang, J. C. (1980) Biochemistry 19, 5229–5234
25. Deleted in proof
26. Riggs, A. D., Suzuki, H., and Bourgeois, S. (1970) J. Biol. Chem. 244, 450–459
27. Cairns, J. (1963) J. Mol. Biol. 6, 208–213
28. Changsoux, J. J., and Been, M. D. (1980) in Mechanistic Studies of DNA Replication and Genetic Recombination (Alberts, B., ed) pp. 809–815, Academic Press, Inc., New York
29. DiGate, R. J., and Marians, K. J. (1988) J. Biol. Chem. 263, 13366–13373
30. Kim, R., and Wang, J. C. (1992) J. Biol. Chem. 267, 17178–17185
31. Kornberg, A., and Baker, T. A. (1992) DNA Replication, 2nd ed., pp. 379–401, W. H. Freeman Co., New York
32. Zechiedrich, E. L., and Cozzarelli, N. (1995) Genes & Dev. 9, 2859–2869
33. Yamagishi, J., Furutani, Y., Inoue, S., Ohsue, T., Nakamura, S., and Shimizu, M. (1981) J. Bacteriol. 140, 450–459
34. Khodursky, A. B., Zechiedrich, E. L., and Cozzarelli, N. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11801–11805

2 H. Hiasa and K. J. Marians, unpublished observations.
