To further define the nucleic acid determinants that govern the recognition of DNA by topoisomerase II, the ability of the enzyme to cleave a 51-base oligonucleotide that contained a centrally located 19-base hairpin was characterized. Topoisomerase II cleaved the 51-mer in a site-specific fashion, within the hairpin, one nucleotide from the 3'-base of the stem. Protein denaturants were not required to trap cleavage products. Although the sequence of the oligonucleotide influenced levels of enzyme-mediated DNA scission, it did not affect the spatial location of cleavage. DNA scission required a double-stranded/single-stranded junction at the 3'-base of the hairpin and a tail (either single- or double-stranded) at least 8 bases in length on the 5'-side. Cleavage was not observed when base-pairing within the oligonucleotide was eliminated or when the hairpin was extended to produce a completely double-stranded substrate. Finally, the enzyme displayed a size constraint for both the stem and loop structures of the hairpin. These results indicate that topoisomerase II is capable of recognizing secondary structure within nucleic acids and identifies the first secondary structure-specific DNA recognition/cleavage site for the type II enzyme.

Topoisomerase II is a highly conserved enzyme (1, 2) that plays critical roles in chromosome structure (3–7) and function (8–20). The enzyme modulates the topological state of DNA in eukaryotic cells by passing an intact nucleic acid helix through a transient double-stranded break made in a second helix (21–23). During its scission reaction, topoisomerase II maintains the topological integrity of its nucleic acid substrate by forming covalent phosphotyrosine bonds with both newly generated 5'-DNA termini (24–28).

Type II topoisomerases share the ability to cleave and religate DNA with a number of enzymes that mediate site-specific recombination such as λ Int (29–31), P1 Cre (29, 32), and yeast Flp (29, 33, 34). Consequently, in addition to its physiological roles in regulating homologous DNA recombination and resolving recombination products (13, 35, 36), topoisomerase II also is believed to mediate nonhomologous (illegitimate) recombination events in the cell (37). Several lines of evidence support this hypothesis. First, introduction of exogenous topoisomerase II into mouse cells greatly increases the frequency of recombination (38). Second, sites of in vivo recombination often correlate with sites of DNA cleavage mediated by topoisomerase II (39–42). Third, anionic drugs that stabilize covalent topoisomerase II-cleaved DNA complexes promote recombination, mutagenesis, and chromosomal translocations in treated cells and patients (43–54). Finally, the eukaryotic type II enzyme mediates illegitimate recombination in vitro (55). Thus far, two models for topoisomerase II-mediated nucleic acid recombination have been proposed. They are the subunit exchange (37, 55, 56) and the intermolecular ligation (57, 58) models, respectively. The subunit exchange model requires that two independent enzyme homodimers simultaneously introduce double-stranded breaks in the backbones of separate DNA helices (37, 55, 56). Recombination is mediated by the exchange of subunits between the two homodimers followed by ligation to generate new DNA species. In this model, the 3'-hydroxyl of the cleaved DNA (which is not covalently attached to the enzyme) never dissociates from the active site of topoisomerase II.

In contrast to the above, the intermolecular ligation model requires the action of only a single homodimer of the type II enzyme (57, 58). In this latter model, one of the 3'-hydroxyl DNA termini generated by topoisomerase II-mediated cleavage dissociates from the active site of the enzyme and is replaced by a separate nucleic acid terminus. Recombination is mediated by the nucleophilic attack of the invading 3'-hydroxyl on the enzyme-bound 5'-phosphate terminus and subsequent ligation of the two DNA molecules. The dissociation event that is critical to this model is rare when double-stranded nucleic acid substrates are cleaved by topoisomerase II (55, 58). However, dissociation occurs frequently when molecules with double-stranded/single-stranded junctions (59), DNA duplexes containing nicks, or single-stranded DNA molecules with the potential to form secondary structures (57, 58) are utilized by the enzyme.

The fact that the DNA structures described above exist in vivo (60, 61) and are often located at sites of nucleic acid recombination (62–65) suggests that the intermolecular ligation model for topoisomerase II-mediated DNA recombination may have physiological relevance. Therefore, in order to detail the mechanism by which this potential recombination pathway occurs, interactions between the type II enzyme and a DNA hairpin-containing oligonucleotide were characterized. Results indicate that topoisomerase II cleaves the DNA hairpin in a site-specific fashion and that the 3'-DNA terminus generated by scission is able to diffuse from the active site of the enzyme. Furthermore, the secondary structure rather than the sequence of the oligonucleotide is the dominant feature recognized by the type II enzyme. The substrate characterized in the present study represents the first example of a DNA recognition/cleavage site for topoisomerase II that is dictated
by secondary structure and indicates that nucleic acid hairpins may be targets for recombining events mediated by the type II enzyme.

EXPERIMENTAL PROCEDURES

Topoisomerase II was purified from 6- to 12-h-old Drosophila melanogaster embryos by the procedure of Shelton et al. (66). Terminal cleavage of DNA was determined by the method of diethylpyrocarbonate (DEPC) treatment (65) unless otherwise noted. Oligonucleotide ligation was assessed by gel electrophoresis in 8% polyacrylamide/7 M urea gels, extracted overnight in 500 μl of 10 M Tris-HCl, pH 6.8, 0.1% SDS. Following electrophoresis, DNA was visualized by autoradiography as described in the preceding section. Alternatively, DNA cleavage reactions treated with or without proteinase K were monitored by electrophoresis on a denaturing 14% polyacrylamide gel. DNA cleavage products were visualized by autoradiography.

RESULTS

Previous studies demonstrated that the type II topoisomerase from bacteriophage T4 (67) or D. melanogaster (57, 58) was able to cleave φX174 (+)-strand DNA and that cleavage intermediates could be trapped even in the absence of protein determinants. Although the viral DNA is single-stranded in nature, it has considerable potential for forming secondary structures. Since strong sites of topoisomerase II-mediated DNA cleavage in the φX174 viral strand often map in the vicinity of sequences that form hairpins (67), it is likely that the enzyme has the capability to recognize specific structures in nucleic acids. Therefore, to characterize interactions between topoisomerase II and regions of DNA secondary structure, the ability of the Drosophila enzyme to cleave a hairpin-containing oligonucleotide was assessed.

The DNA substrate employed in this study is shown in Fig. 1. 1. The oligonucleotide is 51 bases long and contains an 8-base pair stem, a 3-base single-stranded loop, and 16-base single-stranded tails on both the 5’- and the 3’-ends of the hairpin. The sequence of the oligonucleotide represents bases 3941-3991 of Drosophila melanogaster DNA (68). This region of the viral DNA is thought to play an important role in transcription termination (69). Furthermore, sites of topoisomerase II-mediated DNA cleavage (as determined by high resolution mapping) appear to be located near this sequence (67).

Cleavage of the Hairpin-Containing Oligonucleotide by Topoisomerase II—Topoisomerase II cleaved the hairpin-containing 51-mer in a site-specific fashion (Fig. 2). When the DNA substrate was labeled with 32Pphosphate on its 5’-terminus, a unique 34-mer was generated following incubation with the enzyme. The length of the reaction product was determined by comparison with oligonucleotide standards derived from the 5’-terminus of the 51-mer (Fig. 2) and was confirmed by co-migration with the predicted 34-mer (not shown). As denoted by the arrow in Fig. 1, the sequence position of topoisomerase II-mediated DNA cleavage is located within the hairpin region of the 51-mer, one nucleotide from the 3’-end of the base.
Under the conditions employed, ~8% of the initial substrate was cleaved following a 30-min incubation with the enzyme.

To further characterize cleavage of the 51-mer by topoisomerase II, alterations were made in the reaction conditions. For comparison, relative levels of DNA cleavage were set to 1.0 for complete 30-min assays. As seen in Table I, cleavage required the presence of topoisomerase II and a divalent cation. Although less efficient than magnesium, calcium (which supports cleavage of double-stranded substrates (70)) also supported scission of the 51-mer. Cleavage of the hairpin did not require ATP and was diminished when topoisomerase II was incubated with the nonhydrolyzable ATP analog, APP(NH)P, prior to the addition of oligonucleotide. This latter finding is consistent with the fact that nonhydrolyzable ATP analogs induce a conformational change that impedes DNA substrates from entering the active site of the enzyme (71, 72).

When double-stranded DNA substrates are employed, protein denaturants such as SDS are required to trap cleavage intermediates (24, 25, 70). No DNA breakage is observed when reactions are terminated by the addition of EDTA or salt (24, 25, 70). In contrast, when substrates such as dXYT4 (+)-strand DNA are employed, cleavage intermediates are observed even in the absence of protein denaturants (57, 58, 67). This is due to the fact that the 3'-hydroxyl termini generated by cleavage of the viral DNA strand can dissociate from the active site of topoisomerase II and uncouple the cleavage/religation equilibrium of the enzyme (57). The DNA cleavage reactions shown in Fig. 2 and Table I were terminated by the addition of EDTA. This finding strongly suggests that the 3'-hydroxyl of the 34-mer product can dissociate from topoisomerase II upon cleavage. This hypothesis was confirmed by several experiments. First, no reversal of DNA cleavage was observed when reactions were treated with salt (500 mM NaCl) prior to EDTA (Table I). Second, levels of the 34-mer cleavage product that were 3' labeled following a 30- or 60-min cleavage assay (open bars). Data are plotted as the percentage of initial DNA substrate.

could be labeled by the addition of terminal deoxynucleotidyltransferase and [α-32P]dATP (Fig. 3, inset). Following either a 30- or 60-min cleavage assay, ~75% of the 34-mer generated by scission of the hairpin-containing oligonucleotide was labeled. No 3'-labeling of the 34-mer was observed when topoisomerase II was absent from reaction mixtures.

Although a protein denaturant was not required to trap DNA cleavage products, higher levels (~2-fold) of 34-mer were observed in reactions that were terminated by the addition of SDS (Table I). Thus, release of the newly generated DNA 3'-hydroxyl appears to be less efficient than the cleavage event. It is likely that topoisomerase II undergoes more than one round of cleavage/religation with the 51-mer before the 34-mer cleavage product dissociates from the active site of the enzyme. This suggestion is supported by the fact that treatment of reactions with salt prior to SDS reduced levels of DNA cleavage to those observed in reactions terminated with EDTA (Table I).

| Reaction | Relative DNA cleavage |
|----------|-----------------------|
| Complete reaction | 1.0 ± 0.08 |
| Topoisomerase II | 0 ± 0 |
| -MgCl₂ | 0 ± 0 |
| +CaCl₂ | 0.6 ± 0.02 |
| +ATP | 1.0 ± 0.06 |
| +APP(NH)P | 0.4 ± 0.02 |
| Terminated with salt | 1.0 ± 0.04 |
| Terminated with SDS | 2.2 ± 0.16 |
| Terminated with salt, then SDS | 1.0 ± 0.02 |

a Unless noted otherwise, all reactions were carried out as described under "Experimental Procedures" and were terminated by the addition of EDTA.
b Relative levels of cleavage in complete reaction mixtures were set to 1.0. Data are the averages of three independent experiments. Standard deviations are indicated.

c A concentration of 5 mM CaCl₂ was employed in place of MgCl₂.

d A concentration of 1 mM APP(NH)P was employed.

e Sodium chloride (500 mM final concentration) was added, and reactions were incubated at 30 °C for 5 min prior to the addition of EDTA.

f SDS (1% final concentration) was added to reactions prior to EDTA.

g Sodium chloride (500 mM final concentration) was added and reactions were incubated at 30 °C for 5 min, then SDS (1% final concentration) was added prior to EDTA.
Effects of Nucleotide Sequence on Cleavage of the Hairpin-containing Oligonucleotide—Sites of topoisomerase II-mediated cleavage in double-stranded DNA are dictated by the nucleotide sequence of the substrate (24, 73–77). In many cases, substitution of even a single base within the nucleic acid recognition sequence of the enzyme abolishes cleavage (77). To examine the effects of primary structure on the ability of topoisomerase II to cleave the hairpin-containing oligonucleotide, a series of 51-mers with altered DNA sequences was synthesized. It should be noted that the secondary structure of the parent oligonucleotide was maintained throughout this series.

The first oligonucleotide examined was one in which the sequence of the entire 51-mer was inverted (i.e. the sequence that read 5'- to 3' was complemented). As seen in Figs. 5 and 6, topoisomerase II-mediated cleavage of the inverted oligonucleotide was about 6-fold lower than that observed with the parent substrate. Remarkably, however, the spatial location of DNA cleavage (i.e. one nucleotide from the 3'-base of the hairpin and 34 bases from the 5'-terminus) was identical with that obtained with the parent 51-mer (Fig. 5). No cleavage was observed at any other sequence position.

To further assess the effects of DNA sequence on the cleavage of the oligonucleotide by topoisomerase II, three additional 51-mers were constructed; the first inverted the 2 bases directly flanking the cleavage site, the second inverted the hairpin (i.e. stem/loop) sequence, and the third replaced the single-stranded tails with their complementary sequences (Fig. 6). As above, the spatial location of cleavage for all three oligonucleotides was identical with that obtained in the parent 51-mer. However, the primary structure of the oligonucleotides influenced levels of enzyme-mediated DNA cleavage. Inversion of the 2 bases directly flanking the cleavage site decreased levels of cleavage ~2.5-fold. Inversion of the stem/loop sequence had a greater effect, decreasing DNA scission to a level comparable to that of the inverted 51-mer. Finally, the sequence of the single-stranded tails had little effect on topoisomerase II-mediated DNA cleavage.

These results indicate that the DNA sequence of the 51-mer influences levels of topoisomerase II-mediated DNA scission, with alterations in the vicinity of the cleavage site having the greatest effect. However, since DNA sequence did not affect the spatial location of cleavage, this suggests that the site specificity of the enzyme for this hairpin is dictated by the secondary, rather than the primary structure of the oligonucleotide.

Requirement for a Double-stranded/Single-stranded DNA Junction in the Oligonucleotide—Since the site of cleavage by topoisomerase II is one base from the double-stranded/single-stranded DNA junction on the 3'-side of the hairpin, the requirement for this junction in enzyme recognition/scission was characterized (Fig. 7). When base-pairing within the hairpin was eliminated by converting the bases in the 5'-half of the stem to their complements, cleavage of the oligonucleotide was abolished. An identical result was obtained when the hairpin-containing substrate was replaced by two oligonucleotides that made up the 5'- and 3'-halves of the 51-mer but were not

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**Fig. 4. Covalent attachment of topoisomerase II to the 5'-DNA terminus generated by cleavage of the hairpin-containing 51-mer.** A 5'-radiolabeled oligonucleotide was employed for this experiment. Results were monitored by electrophoresis on a denaturing agarose gel. Topoisomerase II was visualized by staining with Coomassie Brilliant Blue (left panel), and labeled DNA was visualized by autoradiography (right panel). The position of a topoisomerase II standard is indicated. Lanes 1, topoisomerase II was added to reactions following the addition of EDTA and SDS (i.e. no cleavage took place); lanes 2, topoisomerase II was present in reactions for 30 min prior to the addition of EDTA and SDS (i.e. cleavage occurred).

**Fig. 5. Cleavage of the inverted hairpin-containing 51-mer by topoisomerase II.** An autoradiogram of a polyacrylamide gel is shown. The positions of 51- and 34-base oligonucleotide standards are indicated. Topoisomerase II-mediated cleavage of the parent 51-mer (lane 1, 51mer) and the inverted 51-mer (lane 2, INV) are shown.

**Fig. 6. Effects of nucleotide sequence on the cleavage of the hairpin-containing oligonucleotide.** Relative levels of DNA cleavage are shown for: the parent oligonucleotide (51mer); an oligonucleotide in which the parent sequence was inverted from 5'→3' to 3'→5' (INV 51mer); an oligonucleotide in which the 2 base pairs that immediately flank the site of cleavage were inverted (i.e. the sequence at the point of cleavage was changed from 5'→3' to 3'→5') (INV Site); an oligonucleotide in which the hairpin (i.e. stem/loop) region was inverted from 5'→3' to 3'→5' (INV HP); and an oligonucleotide in which the single-stranded DNA tails were replaced with tails whose sequences were complementary to those of the parent substrate (cTails). Data represent the averages of 2-3 independent experiments. Standard errors or deviations (as appropriate) are indicated. The relative level of DNA cleavage for the parent 51-mer was set to 1.0.
Fig. 7. Requirement for a double-stranded/single-stranded DNA junction in the oligonucleotide. Relative levels of DNA cleavage are shown for: the parent oligonucleotide (51-mer); a mix of a 24-mer and a 27-mer (i.e. the 5'- and 3'-halves of the parent substrate) that were not annealed (24-mer + 27-mer); a single-stranded oligonucleotide in which the hairpin was eliminated by converting the bases in the 5'-half of the stem to their complements (SS); and a double-stranded oligonucleotide in which the double-stranded/single-stranded DNA junction was eliminated by replacing the sequence of the 3'-tail to one that was complementary to the 5'-tail (DS). Data represent the averages of 3-5 independent experiments. Standard errors or deviations (as appropriate) are indicated. For the parent 51-mer, data were set to 1.0.

annealed. These results indicate that base pairing is essential for topoisomerase II-mediated cleavage of the parent oligonucleotide. Finally, no cleavage was observed when the double-stranded/single-stranded DNA junction was eliminated by converting the sequence of the 3'-tail to one that was complementary to the 5'-tail (DS). Data represent the averages of 3-5 independent experiments. Standard errors or deviations (as appropriate) are indicated. For the parent 51-mer, data were set to 1.0.

Increasing the length of the double-stranded DNA stem had a dramatic effect on the efficiency of enzyme-mediated scission (Fig. 8). Adding even 2 base pairs to the top of the stem decreased cleavage by >90%. Moreover, adding 4 base pairs nearly eliminated cleavage. Decreasing the length of the stem by removing the 2 base pairs adjacent to the loop had a lesser effect on DNA scission. When this oligonucleotide with a 6-base pair stem was employed, the level of DNA cleavage was ~70% that of the parent hairpin. It is not clear whether this latter effect is due to an altered interaction of the oligonucleotide with the enzyme or to a diminished stability of the shortened stem.

These results suggest that topoisomerase II has a size constraint for its recognition of the hairpin. If this suggestion is correct, increasing the size of the loop could also adversely affect the ability of the enzyme to cleave the oligonucleotide. As shown in Fig. 7, this was the case. When the 3-base loop of the parent 51-mer was replaced by a 6- or 9-base loop, levels of cleavage dropped by ~84% or ~96%, respectively. Thus, it appears that topoisomerase II recognizes not only the double-stranded/single-stranded junction of the oligonucleotide, but the size of the hairpin as well.

Requirement for the Single-stranded DNA Tails of the Oligonucleotide—Footprinting data indicate that topoisomer-
When the 15-mer was replaced by a 12-base complementary isomerase I1 mediates recombinational events within the cell DNA scission was restored to that of the parent 51-mer. Therefore, it appears that topoisomerase I1 requires the DNA flanking the 3'-side of the hairpin to be single-stranded for at least 2–4 nucleotides.

**DISCUSSION**

Considerable circumstantial evidence suggests that topoisomerase II mediates recombinational events within the cell (37). In vitro, nucleic acid molecules with the potential to form secondary structures are efficient substrates for topoisomerase II-mediated DNA cleavage (57, 58, 67) and intermolecular ligation (57, 58). Considering that such DNAs are often hot spots for illegitimate recombination within the genetic material (62–65), and that hairpins are intermediates in the recombination of V(D)J regions (67), interactions between topoisomerase II and a hairpin-containing DNA oligonucleotide were examined. Topoisomerase II cleaved the hairpin in a site-specific fashion, one nucleotide from the 3'-base of the stem. Furthermore, the 3'-hydroxyl generated by DNA cleavage was able to dissociate from the active site of the enzyme. Therefore, DNA hairpins are potential substrates for topoisomerase II-mediated illegitimate recombination.

Little is known about the features that govern the intrinsic site specificity of topoisomerase II for its nucleic acid target. Although the topological state of DNA (80), the presence of ATP or nonhydrolyzable ATP analogs (24, 81, 82), and the substitution of other divalent cations for magnesium (28, 70) all influence the efficiency of enzyme-mediated cleavage, none of these affects the site of nucleic acid scission. All currently available evidence indicates that topoisomerase II binding (78, 79) and cleavage (24, 73–77) are directed by the primary structure of its DNA substrate. However, while several consensus nucleic acid sequences for cleavage have been proposed, they bear little resemblance to one another (24, 73–77). Thus, the precise sequence determinants that dictate site specificity still remain equivocal.

To date, all DNA cleavage sites identified for topoisomerase II are double-stranded in nature (21–23). Although sites of DNA cleavage have been described in substrates that contain double-stranded/single-stranded junctions (59), topoisomerase II also cuts these sites when they are located in completely double-stranded regions (24, 59, 83). The DNA hairpin described in the present study represents a unique class of recognition sites for topoisomerase II-mediated cleavage. The enzyme does not utilize this site when it is embodied in purely single-stranded or double-stranded DNA. Furthermore, in marked contrast to sites of double-stranded DNA scission, site specificity within the hairpin is not influenced by nucleic acid sequence. Finally, in addition to its requirement for a double-stranded/single-stranded DNA junction, the enzyme also recognizes the size of both the stem and the loop structures. Thus, topoisomerase II must be interacting with regions of the hairpin that are distal to the spatial location of cleavage.

In conclusion, the present study demonstrates that topoisomerase II is capable of recognizing secondary structure within nucleic acids and identifies the first secondary structure-specific DNA recognition/cleavage site for the type II enzyme.

**REFERENCES**

1. Wyckoff, E., Natalie, D., Nolan, J., Lee, M., and Hsieh, T. (1989) J. Mol. Biol. 205, 1-13
2. Huang, W. M. (1990) in DNA Topology and Its Biological Effects (Costantelli, N., R., and Wang, J. C., eds) pp. 409–457, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
3. Earnshaw, W. C., Halligan, B., Cooke, C. A., Heck, M. S., and Liu, L. F. (1985) J. Cell Biol. 100, 1706–1715
4. Earnshaw, W. C., and Heck, M. M. (1985) J. Cell Biol. 100, 1716–1725
5. Berrios, M., Osheroff, N., and Fisher, P. A. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 4142–4146
6. Gasser, S. M., and Laemmli, U. K. (1986) EMBO J. 5, 511–518
7. Gasser, S. M., LaRoche, T., Falquet, J., Boy de la Tour, E., and Laemmli, U. K. (1986) J. Mol. Biol. 188, 613–629
8. DeLisi, C., Vael, D., and Sternglanz, R. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 2615–2620
9. Uemura, T., and Yagita, M. (1984) EMBO J. 3, 1737–1744
10. Holm, C., Goto, T., Wang, J. C., and Batatan, D. (1985) Cell 41, 553–563
11. Uemura, T., Yagita, M., and Yanagida, M. (1986) EMBO J. 5, 1003–1010
12. Uemura, T., Okamura, H., Adachi, Y., Morino, K., Shiozaki, K., and Yanagida, M. (1987) Cell 60, 917–923
13. Rose, D., Thomas, W., and Holm, C. (1990) Cell 60, 1009–1017
14. Newport, J. (1987) Cell 42, 205–217
15. Newport, J. (1989) Cell 58, 229–236
16. Wood, E. R., and Earnshaw, W. C. (1990) J. Cell Biol. 111, 2839–2855
17. Adachi, Y., Luke, M., and Laemmli, U. K. (1991) Cell 64, 137–148
18. Hirano, T., and Mishcovitch, T. J. (1991) J. Cell Biol. 115, 1479–1489
19. Shamu, C. E., and Murray, A. W. (1992) J. Cell Biol. 117, 821–834
20. Hirano, T., and Nitschow, T. J. (1993) J. Cell Biol. 120, 601–612
21. Wang, J. C. (1985) Annu. Rev. Biochem. 54, 665–697
22. Osheroff, N. (1989) Pharmacol. Ther. 41, 223–241
23. Osheroff, N., Zechiedrich, E. L., and Kalle, C. K. (1991) BioEssays 13, 265–275
24. Sander, M., and Hsieh, T. (1985) J. Biol. Chem. 260, 8421–8426
25. Le, P., Cow, P., and Wang, J. C. (1989) J. Biol. Chem. 264, 4412–4416
26. Zechiedrich, E. L., Christensen, K., Andersen, A. H., Westergaard, O., and Osheroff, N. (1989) Biochemistry 28, 6229–6236
27. Zechiedrich, E. L., Christensen, K., Andersen, A. H., Westergaard, O., and Osheroff, N. (1989) Biochemistry 28, 6229–6236
28. Landy, A. (1989) Annu. Rev. Biochem. 58, 915–949
29. Franz, B., and Landy, A. (1990) J. Mol. Biol. 215, 523–535
30. Eckolds, N. (1990) J. Biol. Chem. 265, 14697–14700
31. Dale, E. C., and Ow, D. W. (1990) Gene (Amst.) 81, 79–85
32. Cox, M. M. (1989) in Mobile DNA (Berg, D. E., and Howe, M. M., eds) pp. 661–670, American Society for Microbiology, Washington, D. C.
33. Craig, G. N. L. (1988) Annu. Rev. Genet. 22, 77–105
34. Christman, M. P., Dietrich, F. S., and Fink, G. R. (1988) Cell 56, 413–425
35. Wang, J. C., Caron, P., and Kim, R. A. (1990) Cell 62, 403–416
36. Dillehay, L. E., Jacobson-Kram, D., and Williams, J. R. (1996) Mutat. Res. 371, 15–23
37. Harford, P. (1991) in DNA Topoisomerases (Potmesil, M., and Berg, D. E., eds) pp. 245–259, Oxford University Press, New York
38. Bullock, P., Champoux, J. J., and Botchan, M. (1985) Science 230, 954–958
39. Pommier, Y., Zwillinger, L. A., Kao-Shan, C.-S., and Bradley, M. O. (1985) Cancer

**FIG. 10. Effects of double-stranded DNA tails on topoisomerase II-mediated cleavage of the oligonucleotide.** Relative levels of DNA cleavage are shown for the parent oligonucleotide (5'1 mer) as well as for 1 mer that was annealed with: a hairpin-containing 51-mer that contained tails that were complementary to the parent substrate (converting it to a cruciform, Crux); a 15-mer complementary to the 5'-tail (5' DS 15bp); a 15-mer complementary to the 3'-tail (3' DS 15bp); and a 12-mer complementary to the 3'-tail (3' DS 12bp). All annealing oligonucleotides were constructed to yield blunt ends at the respective termini of the parent substrate. Data represent the averages of 2 independent experiments. Standard errors are indicated. The relative level of DNA cleavage for the parent 51-mer was set to 1.0.
