Cleavage of DNA by Mammalian DNA Topoisomerase II*

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Using the P4 unknotting assay, DNA topoisomerase II has been purified from several mammalian cells. Similar to prokaryotic DNA gyrase, mammalian DNA topoisomerase II can cleave double-stranded DNA and be trapped as a covalent protein-DNA complex. This cleavage reaction requires protein denaturant treatment of the topoisomerase II-DNA complex and is reversible with respect to salt and temperature. The product after reversal of the cleavage reaction remains supercoiled, suggesting that the two ends of the putatively broken DNA are held tightly by the topoisomerase. Alternatively, the enzyme-DNA interaction is noncovalent, and the covalent linking of topoisomerase to DNA is induced by the protein denaturant. Detailed characterization of the cleavage products has revealed that topoisomerase II cuts DNA with a four-base stagger and is covalently linked to the protruding 5'-phosphoryl ends of each broken DNA strand. Calf thymus DNA topoisomerase II cuts SV40 DNA at multiple and specific sites. However, no sequence homology has been found among the cleavage sites as determined by direct nucleotide-sequencing studies.

Eukaryotic DNA topoisomerase II has been identified in a number of organisms (for reviews, see Refs. 1 to 8). Purified DNA topoisomerase II from eukaryotic cells has enzymatic properties strikingly similar to the bacterial T4-induced DNA topoisomerase, including ATP-(or dATP)-dependent strand-passing reactions and DNA-dependent ATPase activity (9, 10). Different from DNA gyrase, neither T4 DNA topoisomerase nor eukaryotic DNA topoisomerase II can supercoil plasmid DNA in vitro. Whether T4 DNA topoisomerase and eukaryotic DNA topoisomerase II can supercoil DNA in vivo is still unknown. It is clear, at least in bacteria, that DNA topoisomerases control DNA supercoiling which in turn regulates many vital genetic processes (5, 11, 12). In order to study the regulation of the topological structures of eukaryotic DNA, we have purified DNA topoisomerase II from several mammalian cells. We report here our studies of the interaction between mammalian DNA topoisomerase II and DNA.

MATERIALS AND METHODS

Enzymes and Nucleic Acids—HeLa DNA topoisomerase II was purified according to the published procedure (13). Calf thymus DNA topoisomerase II was purified to homogeneity by a similar procedure, except that the P4 unknotting assay was used to monitor the enzyme activity (14). HeLa DNA topoisomerase I, and calf thymus DNA topoisomerase I were purified as the 300 kDa form (15). Most experiments were performed with topoisomerases isolated from both mammalian sources. Plasmid DNA pBR322 dimer was purified by the standard procedure (16). SV40 DNA was purified as described previously (17). All restriction endonucleases and the large fragment of Escherichia coli DNA polymerase I were purchased from Bethesda Research Laboratories. T4 polynucleotide kinase was a gift from Dr. L. Klevan (Bethesda Research Laboratories).

Conditions for Topoisomerase-induced Cleavage of DNA—Cleavage of DNA by mammalian DNA topoisomerase I was done in a reaction mixture (50 µl) containing 10 mM Tris, pH 9, 0.5 mM EDTA, and 10 µg/ml of bovine serum albumin. Cleavage by mammalian DNA topoisomerase II was done in a reaction mixture (50 µl) containing 10 mM Tris, pH 7.0, 1 mM MgCl2, 0.5 mM EDTA, and 10 µg/ml of bovine serum albumin. Appropriate amounts of DNA and topoisomerases were added to initiate the reactions. After 5 min at 37 °C, the reactions were terminated by the treatment with SDS* (1% final).

End Labeling of DNA Restriction Fragments—For labeling of the SV40 restriction fragments, 30 µg of SV40 DNA, digested with HpaII restriction endonuclease, were dephosphorylated with bacterial alkaline phosphatase (18). For 5'-end labeling, half of the sample was treated with T4 polynucleotide kinase and [y-32P]ATP (18). For 3'-end labeling, the other half of the sample was treated with a large fragment of E. coli DNA polymerase I, [5'-32P]dCTP, and unlabelled dCTP at 10 °C for 1 h (18). Unincorporated triphosphates were removed by gel filtration through a G-50 column equilibrated in 0.5 M NaCl, 50 mM Tris, pH 7.7, and 0.5 mM EDTA. Labeled DNA samples were concentrated by ethanol precipitation and then digested with Kpnl restriction endonuclease. End labeling of pBR322 restriction fragments was done similarly.

SDS Precipitation of Topoisomerase-DNA Complexes—(a) SDS precipitation of double-stranded DNA-topoisomerase complexes. Reactions (50 µl each) were terminated by adding 100 µl of a stop solution containing 2% SDS, 2 mM EDTA, and 0.5 mg/ml of salmon sperm DNA, and heated to 65 °C for 10 min. Precipitation of topoisomerase-DNA complex was achieved by the addition of 50 µl of 0.25 M KCl and incubation on ice for 10 min. The precipitate was collected by centrifugation in an Eppendorf centrifuge for 15 min in the cold room. The supernatant was aspirated and the pellet was washed once with 200 µl of a solution containing 10 mM Tris, pH 8.0, 100 mM KCl, 1 mM EDTA, and 100 µg/ml of carrier salmon sperm DNA at 65 °C for 10 min. Following cooling on ice and recentrifugation, the pellet was resuspended in 200 µl of H2O with heating to 65 °C. The suspension was then quickly transferred to a vial containing 4 ml of scintillation fluid (Liquisint, National Diagnostics) and counted. (b) SDS precipitation of single-stranded DNA-topoisomerase complexes. In order to denature the DNA, 0.2 N NaOH was included in the stop solution described above. Heating was done at 37 rather than 65 °C. Precipitation of the topoisomerase-DNA complexes was achieved by the addition of the KCl solution (0.25 M), which was modified to include 0.4 M Tris-HCl in order to neutralize the NaOH. The rest of the procedure was identical with (a).

DNA Nucleotide Sequencing of Topoisomerase II Cleavage Sites—Plasmid DNA pCJ1 was constructed by replacing the EcoRI HindIII (31 bp) fragment of pBR322 DNA with the EcoRI HindIII (74 bp, nucleotide 1692–1769) fragment of SV40 DNA. E. coli MM294 cells were used for transformation. 5'-end labeling of the EcoRI HindIII restriction fragment of SV40 DNA was done as follows. For labeling as the EcoRI site, pCJ1 DNA was cut with EcoRI and labeled with T4 polynucleotide kinase and [y-32P]ATP. Following HindIII digest, the abbreviations used are: SDS, sodium dodecyl sulfate; bp, base pairs.
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RESULTS

Reversibility of the Topoisomerase II Cleavage Reaction—At high concentrations of calf thymus topoisomerase II, cleavage of DNA substrates into the nicked form and the linear form was observed when the reaction mixture was terminated by the addition of SDS to 1% (Fig. 1, lanes A and B). This apparent nuclease activity of calf thymus topoisomerase II, however, is quite different from the classical DNA endonucleases. The cleavage reaction was complete in less than 1 min after incubation at 37 °C. Prolonged incubation did not increase the cleavage product. Furthermore, addition of high concentrations of NaCl (0.25 and 0.5 M NaCl for samples in lanes H and I, respectively) to the reaction mixture after the incubation rapidly reversed the cleavage reaction (compare lanes A, B, H, and I). We have also observed that shifting the temperature from 37 to 0 °C also gradually reversed the cleavage reaction. Both form II and form III plasmid DNA were converted back to form I. This simple salt-induced reversal reaction suggested that cleavage of DNA by topoisomerase II was the result of SDS treatment which trapped a putative intermediate of the topoisomerase reaction. The fact that both form II and form III plasmid DNA were converted back to the supertwisted form but not to the relaxed form further suggested that the two broken DNA ends of the putative enzyme-DNA complex were held tightly by the enzyme. Alternatively, the enzyme-DNA interaction is noncovalent and the covalent linking of enzyme to DNA was induced when the protea denaturant was applied. At this time, we are unable to distinguish between these two possibilities. The require-
ments for this reversible cleavage reaction have been characterized. ATP (or dATP) which is required for the catalytic activity of topoisomerase II, is not required for the cleavage reaction. Contrary to mammalian DNA topoisomerase I which does not show a pH optimum for cleavage, topoisomerase II cleavage reaction shows a sharp pH optimum at about 5. Mg(II) ion stimulates cleavage but is not absolutely required. The cleavage reaction is strongly inhibited by salt above 100 mM NaCl. Single-stranded DNA is also cleaved by topoisomerase II at about the same efficiency. The catalytic reactions of topoisomerase II (e.g. unknotting reaction) are very sensitive to sulfhydryl reagents such as N-ethylmaleimide, while the cleavage reaction is quite resistant to sulfhydryl inactivation. It is possible to inactivate the catalytic activity of mammalian DNA topoisomerase II almost completely, without affecting the efficiency of the cleavage activity, by using sulfhydryl reagents (data not shown).

Covalent Association of Topoisomerase II to Each 5'-end of the Broken DNA Strands—When the topoisomerase II cleavage product was electrophoresed in an agarose gel containing 6.1% SDS (16), the mobilities of both the linear form and the nicked form of plasmid DNA were retarded suggesting that topoisomerase II is still tightly linked to the cleavage products (data not shown). In order to study the cleavage reaction of topoisomerase II more quantitatively, we have developed a procedure which selectively precipitates the protein bound nucleic acids (see “Materials and Methods”). The cleavage product can thus be quantitated by the precipitated counts if the DNA is labeled with radioactivity. Fig. 2 shows such an
experiment. With increasing concentrations of topoisomerase I (Fig. 2, A and B, solid circles), or topoisomerase II (Fig. 2, C and D, solid circles), the radioactivity in the precipitates also increased, suggesting that more covalent topoisomerase-DNA complexes were formed. To confirm that the SDS precipitation procedure only precipitated the covalent topoisomerase-DNA complexes, topoisomerase cleavage products were parallelly quantitated by alkaline agarose gel electrophoresis (discussed in the next section). The electrophoresis results confirmed that the SDS precipitation procedure indeed selectively precipitated DNA molecules which had covalently linked proteins (data not shown). This simple SDS precipitation procedure was also exploited to determine the polarity of the topoisomerase linkage. In this experiment, single-stranded DNA-topoisomerase complexes were precipitated (see “Materials and Methods”) (Fig. 2, triangles). Depending on whether the topoisomerase was linked to the 3' - or 5'-ends of the broken DNA strands and whether 3'- or 5'-end labeled DNA was used, protein-DNA complex may or may not be associated with the labeled ends. A similar analysis has been used to determine the polarity of the E. coli DNA topoisomerase I linkage (19). In the case of mammalian DNA topoisomerase I, labeled protein-DNA complexes were recovered by precipitation only when 5'-end labeled DNA was used (Fig. 2B, triangles). No labeled DNA was precipitated when 3'-end labeled DNA was used (Fig. 2A, triangles), even though protein-DNA complexes did form as revealed by precipitation under neutral conditions (Fig. 2A solid circles). This result strongly suggests that calf thymus DNA topoisomerase I is covalently linked to the 3'-ends of the broken DNA strands, consistent with the previous report (20). In the case of calf thymus DNA topoisomerase II, the opposite was observed (Fig. 2, C and D), suggesting that calf thymus DNA topoisomerase II is covalently linked to the 5'-ends of the broken DNA strands.

Electrophoretic Analyses of the Topoisomerase Cleavage Sites on SV40 DNA—In order to map the cleavage sites, linearized SV40 DNA was uniquely labeled at one end by a procedure described in Fig. 3. SV40 DNA was linearized with HpaII restriction endonuclease and end labeled at either the 3'- or 5'-ends with 32P (see “Materials and Methods”). KpnI restriction endonuclease was then used to clip a 50-bp fragment from one end of the labeled DNA. Since the 50-bp fragment (containing one labeled end) is small enough, no attempt was made to separate it from the 5193-bp fragment which contained the other labeled end. Cleavage of labeled SV40 DNA by topoisomerase I and II was analyzed both by native and alkaline agarose gel electrophoresis (18). Fig. 4 shows the autoradiograms of the topoisomerase cleavage products analyzed by native agarose gel electrophoresis. Very little double-stranded DNA cleavage was observed with calf thymus topoisomerase I using either 3'-end labeled SV40 DNA (lanes B to E) or 5'-end labeled SV40 DNA (lanes F to I). At the highest topoisomerase I concentration, a low level of double-stranded cleavage was observed (lanes E and F). Since topoisomerase I is known to introduce transient single-stranded DNA breaks (1-8), the significance of this double-stranded cleavage sites is not clear. Topoisomerase II, however, efficiently cleaved double-stranded SV40 DNA at multiple sites (3'-end labeled DNA was used in lane J and 5'-end labeled DNA was used in lane K).

In order to determine the single strand breaks generated by calf thymus topoisomerase I and II, the cleavage products were analyzed by alkaline gel electrophoresis (Fig. 5). As expected, topoisomerase I efficiently generated single strand breaks on both 3'-end labeled DNA (lane C) and 5'-end labeled DNA (lane F). The cleavage sites on the complementary strands were completely different, consistent with the notion that topoisomerase I is a monomer and introduces only transient single strand breaks on DNA (1-8). Similarly, when the cleavage products of topoisomerase II were analyzed by alkaline gel electrophoresis, specific cleavage products were observed for both end labeled DNAs. Different from topoisomerase I cleavage sites, some of the cleavage sites generated by topoisomerase II on complementary strands mapped near the same positions (compare lanes J and M). However, the intensities of the cleavage sites on complementary strands were quite different. We have noticed that the ratio of double strand to single strand breaks generated by topoisomerase II varied from preparation to preparation. The basis for this variation is not known.

To investigate whether all the cleavage products have topoisomerases covalently linked to the ends, cleavage products

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**Fig. 3. Strategy of mapping the topoisomerase cleavage sites on SV40 DNA.** The detailed procedure is described under “Materials and Methods.”

**Fig. 4. Native agarose gel electrophoresis of the topoisomerase cleavage products.** End labeled SV40 DNA was used for topoisomerase cleavage reactions. Topoisomerase I concentration in lanes B to D were 0, 0.2, 1.0, and 5.0 μg/ml, respectively. Topoisomerase II concentration in lane J was about 2.5 μg/ml. Labeled SV40 DNA was about 1 μg/ml in each reaction. Lane A is the molecular weight marker (EcoRI digest of the 3'-end labeled SV40 DNA). 3'-end labeled SV40 DNA was used in lanes B to E and lane J. Lanes F to I and lane K were the same as lanes B to E and lane J, respectively, except that 5'-end labeled SV40 DNA was used.
which were not treated with proteinase K were also analyzed by alkaline agarose gel (Fig. 5, lanes D, G, K, and N). When 3'-end labeled DNA was used for topoisomerase I cleavage, omission of proteinase K treatment did not change the mobility of the cleavage products in the gel (Fig. 5, compare lanes C and D). However, all the cleavage products were retarded in gel mobility when 5'-end labeled DNA was used (Fig. 5, compare lanes F and C). This result strongly suggested that all the topoisomerase I cleavage products had proteins covalently linked to the 3'-ends of the broken DNA strands. The same experiment was repeated using calf thymus topoisomerase II (Fig. 5, lanes J, K, M, and N). In this case, the result was totally the opposite. The mobility shift was only observed when the 3'-end labeled DNA was used. We thus concluded that topoisomerase II was covalently linked to each 5'-end at the cleavage sites.

The 3'-ends of the Cleavage Product of Calf Thymus Topoisomerase II Possess Hydroxyl Groups—The 3'-ends of the cleavage products produced by calf thymus DNA topoisomerase II were analyzed by the enzymatic modification using both terminal transferase and E. coli DNA polymerase I. We had shown that the cleavage products of topoisomerase II could be labeled by both the terminal transferase and polymerase I, suggesting that the 3'-ends of the cleavage products possessed hydroxyl groups and were recessed (data not shown). To test whether all the cleavage products had the same 3'-OH ends, the following experiment was performed (Figs. 6 and 7). Topoisomerase II cleavage products (pBR322 DNA was used

FIG. 5. Alkaline agarose gel electrophoresis of the topoisomerase cleavage products. The same described in the legend of Fig. 4, were used for the alkaline agarose gel electrophoresis. Topoisomerase I (Topo I) concentration was 0.2 µg/ml (lanes C, D, F, and G), and topoisomerase II (Topo II) concentration was 2.5 µg/ml (lanes J, K, M, and N). Samples in lanes D, G, K, and N were the same as those in lanes C, F, J, and M, respectively, except that proteinase K treatment was omitted. Lanes B and I were the controls (no enzyme) for the 3'-end labeled SV40 DNA. Lanes E and L were the controls (no enzyme) for the 5'-end labeled DNA. Lanes A and H were the molecular weight marker. The mobility shift (compare lane F with G, and J and K) in alkaline agarose gel is characteristic for covalently linked protein-DNA complexes. The polarity of the topoisomerase linkage can also be determined from this analysis.

FIG. 6. The strategy used to determine the configuration of the 3'-ends of the topoisomerase cleavage products. Procedures for topoisomerase (topo) cleavage and end labeling are described under "Materials and Methods."
as substrate) were labeled with \( [\alpha-^{32}P]dATP \) using the large fragment of *E. coli* DNA polymerase I, cut with EcoRI (Fig. 6A), and then analyzed by native agarose gel electrophoresis (Fig. 7, lane 1B). The cleavage products, which were labeled, appeared as specific bands in the gel (Fig. 7, lane B). To prove that these labeled bands were labeled at the cleavage sites by the polymerase reaction, a different experiment was performed (Fig. 6, panel B). In this experiment, the same plasmid DNA was linearized first with EcoRI, end labeled with polymerase, and then cleaved by topoisomerase II (Fig. 6B, and Fig. 7, lane A). By comparing lanes A and B, it is quite convincing that most, if not all, the cleavage products were labeled by polymerase at the topoisomerase II cleavage sites (considering that only one labeled dNTP was used in the polymerase reaction). Lane C is the same as lane B except that relaxed DNA was used. No significant differences were observed whether superhelical DNA or relaxed DNA was used for topoisomerase II cleavage (compare lanes B and C). We thus conclude that the 3'-ends of the topoisomerase II cleavage products possess hydroxyl groups.

**Nucleotide Sequencing of the Topoisomerase II Cleavage Sites**—In order to sequence the cleavage sites on both complementary strands, a short SV40 DNA fragment (74 bp, from nucleotide 1692-1769) was labeled uniquely at its 5'-ends by T4 polynucleotide kinase (see "Materials and Methods"). Topoisomerase II cleavage sites on the labeled DNA fragments (either labeled at nucleotide 1692 or nucleotide 1769) were determined by DNA sequencing (Fig. 8). The cleavage sites on both complementary strands were schematically shown in Fig. 9. Several interesting features of the cleavage sites were noted. 1) There were more than 12 cleavage sites on this 74-bp fragment. The cleavage efficiency varies greatly from site to site. 2) For each cleavage site, the two cuts on the complementary strands were staggered by four base pairs. Site X was an exception, as we could not locate the cut on the complementary strand. 3) No consensus sequence can be deduced for all the cleavage sites.

**DISCUSSION**

Eukaryotic DNA topoisomerase II has enzymatic activities analogous to bacteriophage T4-induced DNA topoisomerase (10, 21). Our present characterization of the cleavage reaction of topoisomerase II further demonstrated the similarity between prokaryotic and eukaryotic DNA topoisomerase II. Similar to DNA gyrase, mammalian DNA topoisomerase II cuts DNA with a four-base stagger and is covalently linked to the 5'-phosphoryl end of each broken DNA strand (5). Different from DNA gyrase, however, mammalian DNA topoisomerase II can efficiently cleavage DNA in the absence of any added drugs. Similar to other DNA topoisomerases, cleavage of DNA by mammalian DNA topoisomerase II is the result of protein denaturant treatment of the topoisomerase II-DNA complex (5, 22). It is particularly significant that the salt- or temperature-induced reversal of the cleavage reaction gave
products that were superhelical rather than relaxed. This result strongly suggests that the topoisomerase II-DNA complex must be organized in such a way that either the two ends of the broken DNA are tightly bound by the enzyme at all times or the covalent linking of enzyme to DNA occurs only when the protein denaturant was added. It is interesting that ATP is not required for the cleavage reaction. It is possible that the ATPase function is associated with the transport of another DNA segment through this breakage and rejoining site.

We do not know the in vivo significance of this partial reaction of topoisomerase II. The cleavage reaction of eukaryotic topoisomerase II is rather efficient under the physiological conditions, compared with that of the prokaryotic DNA gyrase which is highly dependent on oxolinic acid (3-5). Whether this cleavage reaction of topoisomerase II is related to certain illegitimate recombination processes in eukaryotic cells remains to be tested. The cleavage reaction of topoisomerase II (or I) may also provide a useful way for the mapping of the binding sites. However, we have not yet correlated the cleavage sites with the binding sites.

The biological functions of eukaryotic DNA topoisomerases are still unknown. The similarity between eukaryotic DNA topoisomerase II and prokaryotic DNA topoisomerase II (especially T4 DNA topoisomerase) suggests possible similar functions. It is likely that, similar to prokaryotic DNA topoisomerases, the multiple eukaryotic DNA topoisomerases regulate the topological structure of chromatin and thus control a variety of genetic processes. We have shown that eukaryotic DNA topoisomerase II is capable of catenating SV40 chromatin in vitro. Whether eukaryotic DNA topoisomerase II is capable of supertwisting chromatin in vivo is of fundamental importance to our understanding of the structure and function of chromatin. The effect of DNA supercoiling on transcription in eukaryotic cells has been noted (23). It has been suggested that committed genes in eukaryotic cells may be under negative superhelical tension (24). Whether or not this tensioned state of DNA is controlled by DNA topoisomerases is still not known. Many attempts have been made to demonstrate the supertwisting reaction of eukaryotic topoisomerase II in vitro, and all have failed. It is possible that the supercoiling reaction is intimately related to the wrapping of DNA around the enzyme, as demonstrated in the case of DNA gyrase (25, 26).

REFERENCES

1. Champoux, J. J. (1978) Annu. Rev. Biochem. 47, 449-479
2. Wang, J. C. and Lin, L. F. (1979) in Molecular Genetics (Taylor, J. H., ed), Pt. 3, pp. 65-88, Academic Press, New York
3. Cozzarelli, N. R. (1980) Science (Wash. D. C.) 207, 963-960
4. Cozzarelli, N. R. (1980) Cell 22, 327-328
5. Gellert, M. (1981) Annu. Rev. Biochem. 50, 879-910
6. Wang, J. C. (1981) in The Enzyme (Boyer, P., ed), pp. 331-344, Academic Press, New York
7. Gellert, M. (1981) in The Enzyme (Boyer, P., ed), pp. 345-366, Academic Press, New York
8. Liu, L. F. (1983) CRC Crit. Rev. Biochem. in press
9. Liu, L. F., Liu, C. C., and Alberts, B. M. (1979) Nature (Lond.) 281, 456-461
10. Liu, L. F., Liu, C. C., and Alberts, B. M. (1980) Cell 19, 697-707
11. DiNardo, S., Voelkel, K. A., Sternaglitz, R., Reynolds, A. E., and Wright, A. (1982) Cell 31, 45-51
12. Pruss, G. J., Manes, S. H., and Drlica, K. (1982) Cell 31, 35-42
13. Miller, K. G., Liu, L. F., and Englund, P. T. (1981) J. Biol. Chem. 256, 9334-9339
14. Liu, L. F., Davis, J. L., and Calenzar, R. (1981) Nucleic Acids Res. 9, 2979-2989
15. Liu, L. F., and Miller, K. G. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 3487-3491
16. Halligan, B. D., Davis, J. L., Edwards, K. A., and Liu, L. F. (1982) J. Biol. Chem. 257, 3995-4000
17. Edwards, K. A., Halligan, B. D., Davis, J. L., Niven, N. L., and Liu, L. F. (1982) Nucleic Acids Res. 10, 2565-2576
18. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) in Molecular Cloning, pp. 91-173, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
19. Depew, R. E., Liu, L. F., and Wang, J. C. (1978) J. Biol. Chem. 253, 511-518
20. Champoux, J. J. (1977) Proc. Natl. Acad. Sci. U. S. A. 74: 3800-3804
21. Hsieh, T., and Brutlag, D. (1980) Cell 11, 115-125
22. Liu, L. F., and Wang, J. C. (1979) J. Biol. Chem. 254, 11082-11088
23. Harland, R. M., Weintraub, H., and Mcknight, S. L. (1983) Nature (Lond.) 302, 38-43
24. Weintraub, H. (1983) Cell 32, 1191-1203
25. Liu, L. F., and Wang, J. C. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 2098-2102
26. Liu, L. F., and Wang, J. C. (1978) Cell 15, 979-984

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perhaps specific DNA sequences and/or a missing component(s) might stabilize such a wrapping around eukaryotic topoisomerase II in vivo.

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